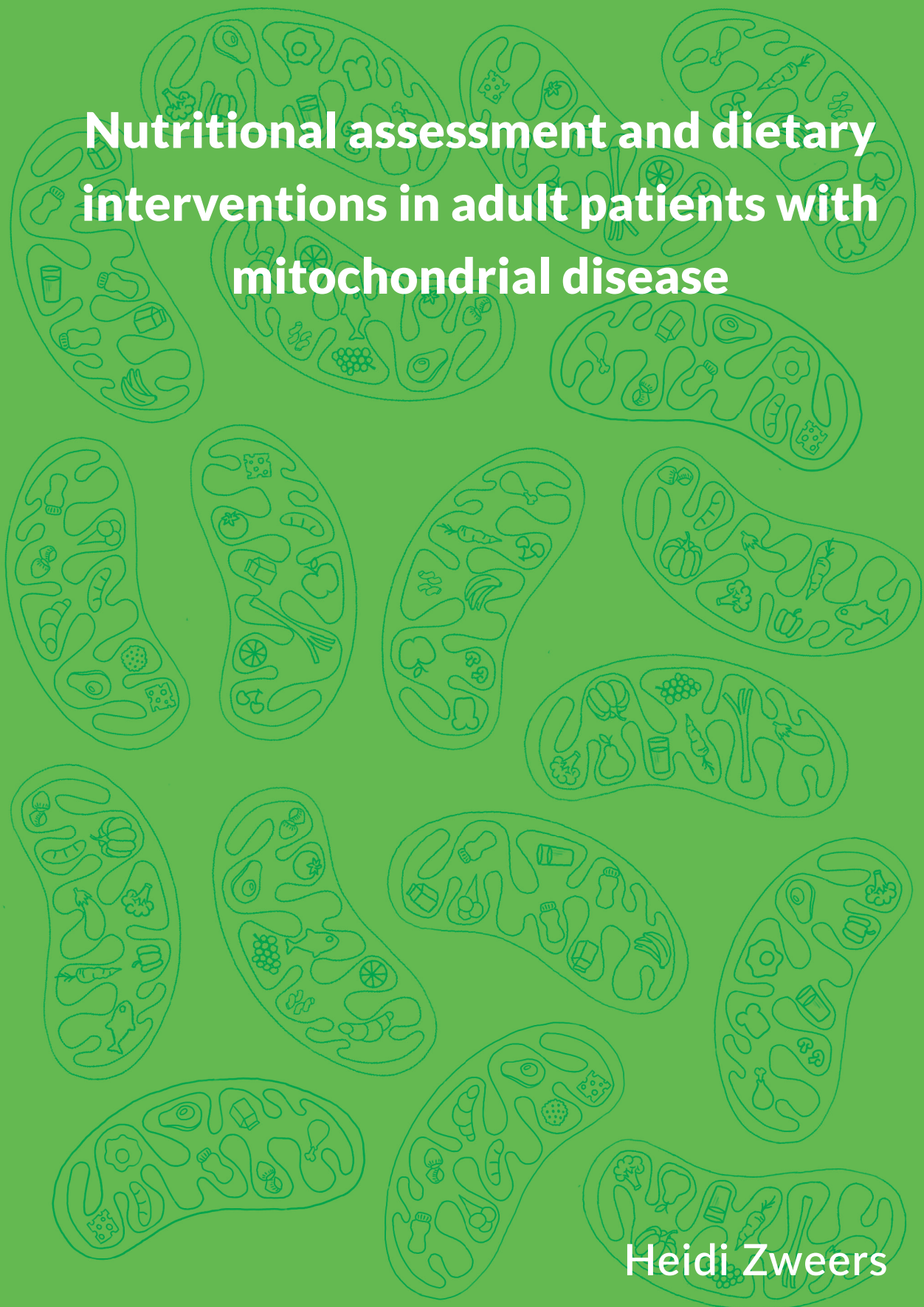
Nutritional assessment and dietary interventions in adult patients with mitochondrial disease



Heidi Zweers

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Nutritional assessment and dietary interventions in adult patients with mitochondrial disease

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Voor mijn trotse ouders

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CHAPTER 1

Introduction

INTRODUCTION

Mitochondrial diseases

The term "Mitochondrial Disease" (MD) coins a group of genetic disorders caused by dysfunctional mitochondria: the organelles that generate energy for the cells. Mitochondria are present in every cell of the human body (except for red blood cells) and convert the energy of nutrients into adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS). MDs are the most prevalent inherited metabolic disorders, with an incidence of approximately 1:5000 live births [1]. MDs are heterogeneous, multi systemic and progressive in nature and may present from infancy to adulthood with a severity ranging from severe during early childhood to a milder phenotype later in life. Tissues with a high energy need, such as the brain, skeletal muscle and heart, are most commonly and severely affected. Maternally Inherited Diabetes and Deafness (MIDD), Mitochondrial Myopathy (MM), and Chronic Progressive External Ophthalmoplegia (CPEO) are the most frequent reported phenotypes in adult patients [2]. Mitochondrial dysfunction can result from mutations in either nuclear DNA or mitochondrial DNA. For mitochondrial DNA mutations the percentage of affected mitochondria per cell in a specific tissue is referred to as heteroplasmy, and the level of heteroplasmy may vary widely between tissues of a single individual. This marked intra-individual and inter-individual variation in mitochondrial heteroplasmy (partially) explains the wide spectrum of diseases and disease severity that can be observed between family members that carry the same mitochondrial mutation. Mitochondrial DNA mutations have maternal inheritance. With the most frequently reported pathogenic type in adults being the m.3243A>G point mutation [2, 3].

The mitochondrial 3243A>G mutation

The m.3243A>G mutation is commonly described as the MELAS mutation. The acronym MELAS was first used by Pavlakis and Phillips [4] to describe a group of patients with Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes. In 1990 the m.3243A>G mutation was found as the molecular basis behind this patient entity [5, 6]. The phenotype Maternally Inherited Diabetes Deafness (MIDD) is also caused by this same mutation and is much more frequent [7]. Other phenotypic expressions of the m.3243A>G mutation include mitochondrial myopathy, hypertrophic cardiomyopathy [8], retinal dystrophy [9], focal segmental glomerulosclerosis [10], myoclonic epilepsy with ragged-red fibers [11] and oligo-symptomatic variants of the acronym MELAS. [12, 13] In the Radboud Centre for Mitochondrial Medicine (RCMM) there is a well-documented cohort of 151 m.3243A>G carriers who are followed in the natural disease course study [14]. The overall progression of disease in carriers of the m.3243A>G mutation is slowly progressive. Heteroplasmy levels and disease severity do not have a significant impact on this progression [14].

Malnutrition in MD

According to the definition by Stratton [15], the term 'disease related malnutrition' coins a shortage and/or disbalance of nutrient intake that leads to negative changes in body composition, functioning, and clinical outcome (Figure 1). By this definition, the energy shortage in MD is malnutrition on a cellular level. Malnutrition is a complex problem and according to the definition of Stratton both undernutrition as well as obesity could be present, and both are known to occur in MD patients [16, 17]. The combination of low muscle mass and low muscle strength seen in malnourished MD patients may also be defined as sarcopenia [18, 19].

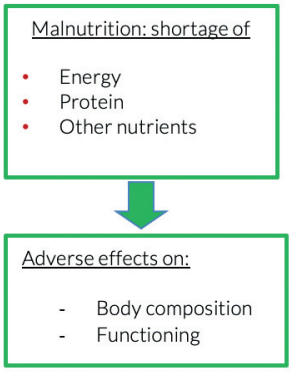

Figure 1. Malnutrition according to Stratton [15].

Body composition is known to be important for physical functioning in the general population and even more in neuromuscular disorders [20-23]. Altered body composition in MD patients has been reported [17, 24] and a higher skeletal muscle mass index in these patients was correlated with higher muscle strength [24]. The low physical functioning in MD patients may also be related to alterations in both nutritional intake and nutritional status. Recent observational studies suggested that MD patients have inadequate protein intake and are at risk of malnutrition [17, 25]. This could affect both body composition and physical functioning. The physical functioning of MD patients appears to be determined by the availability of energy (ATP) on the one hand and the nutritional status on the other. The assumed relationship between these is summarized in Figure 2.

It is suggested that malnutrition may worsen symptoms in MD, as for instance was shown in a study in young MD patients in which an association between nutritional status and mitochondrial functioning was observed [26]. Vice versa, it is known from other diseases such as anorexia nervosa [27] or cancer cachexia [28, 29] that malnutrition per se causes secondary mitochondrial dysfunction. From data of children with mitochondrial disorders it was suggested that by improving the nutritional status in MD patients, ATP production may be improved [30].

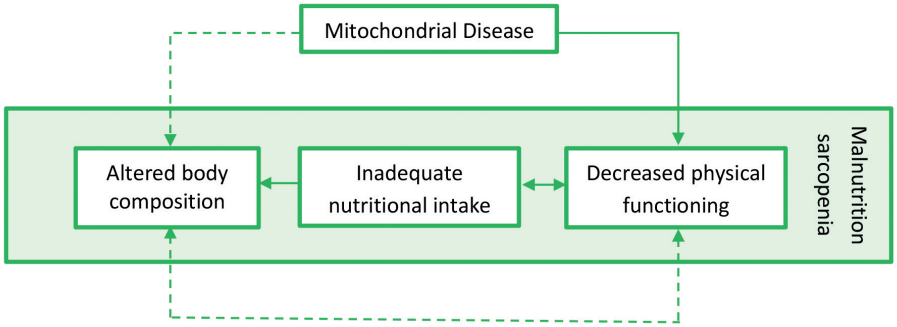

Figure 2. Assumed relationship between body composition, nutritional intake and physical functioning in patients with MD.

Knowledge gap

At this point there is no proof that the presumed associations in Figure 2 do exist, also data on the incidence of malnutrition and sarcopenia in the adult MD population are lacking.

Nutritional Assessment

Nutritional assessment entails the systematic assessment of nutritional status and nutritional needs. Measurements are performed in a structured (subjective and objective) manner that can be categorized into three domains:

1. Food intake and requirements
2. Body composition
3. Functional parameters

These tree domains correspond with the definition of malnutrition [15] (Figure 1). Nutritional assessment is part of every dietary consultation and is essential in dietary diagnostics and -research. The individual dietary treatment plan is based on data from the nutritional assessment. Nutritional assessment as such can be performed on many levels, ranging from very basic to more sophisticated (and accurate) assessments. Basics for example comprise an assessment of dietary history regarding food intake, anthropometry (height and weight measurements) for body composition and interview data for functioning. "More accurate" also implies more time consuming, expensive and invasive tests for patients. In striving for the best possible individual dietary treatment options dietitians search for the optimal balance between intensive assessment methods and obtaining reliable data to design the individual treatment plan.

Diagnosing malnutrition in MD patients is challenging [17, 31] since standard screening tools aim to screen acute malnutrition and are not applicable in MD patients because of chronic malnutrition [25].

Knowledge gap

Because of the very heterogenous phenotypical character of the MD patient population recent literature suggests to perform nutritional assessment to determine the nutritional status [17, 25], yet the most optimal techniques, and the cutoff values in MD are unknown.

Interventions

Until now, no effective treatment for MD is available. Current treatment strategies focus on symptom relief, securing physical functioning and quality of life [3]. It is known that MD patients benefit from exercise interventions [16, 32-37]. MD is a heterogenic disease and the long list of symptoms includes complaints that possibly influence nutritional intake or are influenced by nutrition like; short statue, gastro-intestinal symptoms, dysphagia, fatigue, diabetes and epilepsy [3]. Therefore, in daily practice many nutritional interventions are already common in MD but the evidence to support these interventions is scarce. Examples of such interventions include the ketogenic diet and various vitamin cocktails but also interventions that are commonly used for specific complaints like a high fibre diet in case of constipation.

The Radboud Center for Mitochondrial Medicine (RCMM) has expertise in MDs in general and with individual dietary interventions.

Some of the normal symptom-based interventions that seem effective for other patient categories fail in these patients. For example, it is common in patients with dysphagia to advice thickened liquids. This strategy does not work in MD patients because consuming the thickened liquids requires more energy and leads to fatigue-induced swallowing problems. The evidence of this and similar advises are lacking in the literature so far.

It is difficult to provide solid scientific evidence for positive effects of any intervention on the clinical outcome in the setting of MD, due to the small number of patients and the heterogeneous nature of the population [38]. Therefore, a personalized intervention approach in these patients might be more appropriate.

Knowledge gap

Adequate data on the frequency of dysphagia and gastrointestinal problems and their association with malnutrition in MD patients is lacking so far. The same applies for evidence on personalized nutritional interventions based on nutritional assessment outcomes. More specifically, the evidence on the efficacy and safety of a ketogenic diet as nutritional intervention for genetically proven MD has not been systematically reviewed yet.

AIM OF THIS THESIS

The overall aim of this thesis is to improve the knowledge on nutrition-related issues in MD and thereby improve the functioning and quality of life in adult MD patients.

To this end tree goals are formulated (Figure 3):

1. To gain knowledge on the nutritional status and its determinants in adult MD patients.
2. To define the optimal strategy for nutritional assessment in adult MD patients.
3. To provide evidence for individually tailored nutritional interventions in MD patients.

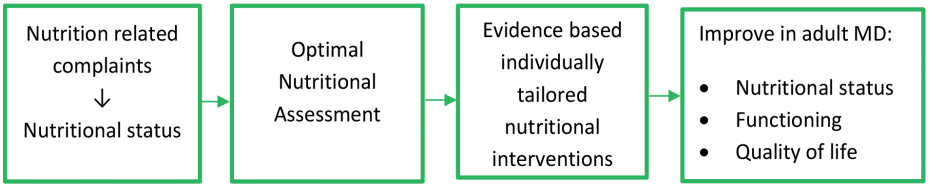

Figure 3. Research aims of this thesis.

APPROACH AND OUTLINE OF THE THESIS

Part 1 focuses on Nutritional Assessment in adult MD patients.

Chapter 2 is an observational study that explores the frequency and severity of gastrointestinal complaints in 92 adult patients carrying the m.3243A>G mutation using a validated questionnaire. Data were compared with those obtained in healthy controls. Furthermore, the association between symptoms, disease severity and the risk for malnutrition and BMI was assessed.
Chapter 3 focuses on the first part of the malnutrition definition: nutritional intake. In this observational, cross-sectional, retrospective study, 60 three-day nutrition diaries of adult MD patients were analysed and compared with the Dutch recommended daily allowance and the Dutch National Food Consumption Survey.
Chapter 4 comprises the results of the DYNAMO[1] study. This study focuses on the associations between physical functioning of MD patients and the alterations in body composition and protein intake (Figure 2). Also, the occurrence of malnutrition and sarcopenia was assessed. Importantly, in this 2-site cross-sectional study, genetically proven adult MD patients were age-, body mass index-, and gender-matched to controls.

1 The Acronym DYNAMO stands for DYnamic (physical functioning) and Nutritional Assessment in MitOchondrial diseases

Chapter 5 aimed to identify the optimal method to estimate total energy expenditure in MD patients. Resting energy expenditure was measured in adult MD patients carrying the m.3243A>G mutation using indirect calorimetry and compared with results of 21 predictive equations for resting energy expenditure, as well as with indirect calorimetry data in healthy controls. Physical activity level (PAL) was assessed using accelerometery (SenseWear®) and compared with a fixed average PAL as well as with patients' self-estimated activity levels. Measured total energy expenditure was compared with usual care and energy recommendations for healthy adults.

Part 2 focuses on Nutritional Interventions in adult MD patients.

Chapter 6 describes the results of the DINAMITE[2] study. The aim of this randomized controlled trial was to explore the effect of an individually tailored dietary intervention on personalized goals, body composition, functioning, and quality of life in 39 adult MD patients due to the m.3243A>G mutation. The intervention group (n = 20) received an individually tailored dietary intervention over a 6-month period, whereas controls (n = 19) received standard care during 6-months (control period), followed by an individually tailored dietary intervention for the next 6 months (intervention period). Nutritional assessment and QoL measurements were performed at 3-month intervals. **Chapter 7** provides a systematic review on the efficacy and safety of the ketogenic diet in genetically proven MD patients (both paediatric and adults).

2 The Acronym DINAMITE stands for DIner Nutristional Assesment MITochondrial disorder Energy

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PART 1

Nutritional Assessment

CHAPTER 2

Dysphagia, malnutrition and gastrointestinal problems in patients with mitochondrial disease caused by the m.3243A>G mutation

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ABSTRACT

Background

Previous research has shown that dysphagia and gastrointestinal problems occur frequently in carriers of the m.3243A>G mutation, however the exact frequency and severity have not been determined. We hypothesize that adult carriers have an increased risk for malnutrition.

Methods

In this observational study we evaluated the presence of gastrointestinal problems and dysphagia in 92 carriers of the m.3243A>G mutation. The severity of the general disease involvement was classified using the Newcastle Mitochondrial Disease Adult Scale (NMDAS). Gastrointestinal involvement, dysphagia and the risk for malnutrition were scored using the Gastrointestinal Symptoms Questionnaire and The Malnutrition Universal Screening Tool. Gastrointestinal symptoms and anthropometrics were compared to healthy controls.

Results

Our results show that height, weight and BMI of these carriers were lower than the national average ($p<0.05$). Seventy-nine carriers (86%) suffered from at least one gastrointestinal symptom, mainly flatulence or hard stool. Both frequency and severity of symptoms were significantly increased compared with reference data of healthy Dutch adults. Forty-five percent of the carriers reported (mostly mild) dysphagia. Solid foods cause more problems than liquids. A negative correlation between BMI and heteroplasmy levels in urinary epithelial cells (UEC) was present (spearman c.c.= - 0,319, p= 0,003).

Conclusion

Dysphagia and gastrointestinal problems, especially constipation are common complaints in the total m.3243A>G carriers cohort and are not related to heteroplasmy levels in UEC or disease severity. The severity of gastrointestinal problems as well as overall disease severity is associated with an increased risk for malnutrition.

INTRODUCTION

Mitochondria and the m.3243A>G mutation

Mitochondrial diseases are the most prevalent inherited metabolic diseases, with an incidence of approximately 1:5000 live births [1]. Mitochondria are the cellular organelles responsible for oxidative phosphorylation, which produces energy in the form of adenosine triphosphate. This process is accomplished by the four complexes (complex I-IV) of the respiratory chain and F1-F0 ATP synthase. Mitochondrial dysfunction can result from mutations in either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA).

The acronym MELAS was first used by Pavlakis and Phillips [2] to describe a group of patients with Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes. In 1990 the m.3243A>G mutation was found as the molecular basis of this disease [3, 4]. The mutation is located in the MT-TL1 gene and is the most common pathogenic mitochondrial mutation [5-7], commonly described as the MELAS mutation. The phenotype maternally inherited diabetes deafness (MIDD) is also caused by this same mutation and is much more frequent [8] Other phenotypic expressions of the m.3243A>G mutation include hypertrophic cardiomyopathy [9], retinal dystrophy [10], focal segmental glomerulosclerosis [11], myoclonic epilepsy with ragged-red fibers [12] and oligo-symptomatic variants of the acronym MELAS [13, 14]. The percentage of affected mitochondria per cell in a specific tissue is referred to as heteroplasmy, and the level of heteroplasmy may vary widely between tissues of a single individual. This marked intra-individual and inter-individual variation in mitochondrial heteroplasmy (partially) explains why there is a wide spectrum of diseases and disease severity observed between family members that carry the same mitochondrial mutation. Previous studies have shown that urinary epithelial cells (UEC) are the best non-invasively available tissue to test the level of heteroplasmy of the m.3243A>G mutation [15, 16].

Gastrointestinal involvement in m.3243A>G carriers

Dysphagia and gastrointestinal problems occur frequently in mitochondrial patients, including patients carrying the m.3243A>G mutation [17-19]. However, the exact frequency and severity of these symptoms have not been determined. In a population study of carriers of the m.3243A>G mutation, 61% of the subjects had gastrointestinal complaints. These were, after hearing loss, the most frequently reported symptoms of the m.3243A>G mutation using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) [20]. The NMDAS is however not a specific instrument to study gastrointestinal problems.

Previously in a small cohort of MIDD patients, a high prevalence of constipation and/or diarrhea (88%) was found [21]. Severe gastrointestinal problems like the pseudo obstruction syndrome, surgery-requiring constipation and pancreatitis have been described in patients carrying the m.3243A>G mutation, although the incidence in these patients remains unclear [18, 21]. Gastrointestinal problems are frequently reported in healthy controls

as well [22], and so far, studies comparing the frequency and severity of gastrointestinal problems in patients with a mitochondrial disease and healthy controls are lacking.

Dysphagia in mitochondrial disease has been described in several studies which are hard to compare because of variations in definitions and study methods. In patient with the m.3243A>G mutation, incidences vary from 18% in a study using the NMDAS to score dysphagia to 38% in a study performed using a more specific approach [23, 24]. Also, short stature and lower bodyweight are frequently described in mitochondrial disease. Patients with a MIDD phenotype have lower body mass index (BMI, weight(kg)^2/height(m)) compared to other diabetic patients [25-27].

For both dysphagia and gastrointestinal problems, a relation with lower body weight and risk for malnutrition in patients with a mitochondrial disease has been suggested but never proven [18, 21]. The present study therefore focused on dysphagia and gastrointestinal problems in a cohort of carriers of the m.3243A>G mutation and identification of symptoms that lead to decreased BMI and increased risk for malnutrition.

MATERIALS AND METHODS

Patients

At the Nijmegen Center for Mitochondrial Disorders (www.ncmd.nl) at Radboud university medical centre 114 adult carriers of the m.3243A>G mutation participate in our cohort study [24]. All participants received the questionnaire. This study was approved by the ethics committee of the Nijmegen-Arnhem region. Written informed consent according to the Helsinki agreement was obtained from all carriers.

General symptoms

All carriers were scored using the NMDAS [28]. The NMDAS constitutes a validated method to monitor the clinical expression of mitochondrial disease and to follow-up the course of disease in time. The NMDAS consists of the following four sections; 1) Current function, which gives insight into the general functioning of patients in past four weeks including swallowing; 2) System-specific involvement, which uses a clinical history supplemented by specific information to gain insight in the functioning of individual organ-systems including gastro intestinal symptoms; 3) Current clinical assessment, i.e. a general and neurological clinical examination, which gives insight in the current functional status of the patient; 4) For quality of life, we used a Dutch translation of the SF-12v2 quality of life test.

Gastrointestinal symptoms evaluation

All participants received the Gastrointestinal Symptoms Questionnaire [29, 30]. This self-report questionnaire contains 16 items regarding gastrointestinal involvement

of disease for the past four weeks. Severity of the symptoms is scored on a 7-point likert scale, where 0 resembles no symptoms and 6 resembles extreme symptoms. The final question of the questionnaire is a 50-point scale asking the overall burden of the gastrointestinal complaints. We added specific questions on dysphagia and frequency of stools. The validated Malnutrition Universal Screening Tool (MUST) [31] was used to collect the self-reported anthropometric data and to screen for the risk for malnutrition. We compared the data to age matched anthropometric data from the Dutch Central Bureau of Statistics (CBS) 2011 (n= 2034) and to a Dutch reference database form the Gastrointestinal Symptoms Questionnaire (n=1616). Data collection for this reference database was done in 2006 participants who matched the general Dutch population were selected with CBS statistics [22].

Mutation Analysis

Heteroplasmy levels of the m3243A>G mutation were determined in UEC in all participants using Pyrosequencing TM technology (Pyrosequencing, Uppsala, Sweden) as earlier described [11]. The pyro sequence reaction of the m.3234A>G mutation had a precision of 1,5%, and the mutation was detected from a heteroplasmy level of 5%. The detection limit for the mutation was determined by serial dilution of a sample containing this mutation with wild type mtDNA.

Statistics

We used descriptive statistics to present the heteroplasmy levels in our patients. Means are presented with their standard deviation. To calculate the significance of the different variables in relation to BMI and gastrointestinal complaints Independent samples T-test was used. We corrected for multiple testing using the Bonferroni test. Pearson correlation coefficient was used to evaluate the relationship between severity of gastrointestinal problems, and BMI. Spearman correlation coefficient was used to evaluate the relationship between heteroplasmy in UEC, gastrointestinal problems, and BMI. We used the pearson chi-square to compare BMI category for males and females and carriers with healthy controls.

RESULTS

General patient characteristics and anthropometrics

Data were collected from September to November 2011. From 114 questionnaires that were sent out, 92 were returned (81% response rate). Of these 92 patients 68% (n=63) were female. Mean age was 45 years (±14.3). In the Gastrointestinal Symptoms Questionnaire database Mean age was 52.3 ± 17.2, 66% (n=1067) were female.

Mean NMDAS score was 15.7 (±10.9) range 0-56 and average heteroplasmy level in UEC was 50% (range 5%-98%). Thirty-eight patients (41%) were diagnosed with diabetes, of

whom 22 (24%) used insulin. Hearing loss was reported by 59 patients (64%), 27 patients (29%) had a hearing aid. Six patients (6.5%) had severe neurological symptoms, of which five (5.4%) suffered from epilepsy and two (2.2%) had had stroke like episodes in the past year.

Table 1. Anthropometrics in male and female m.3243A>G carriers and healthy 40-50 years old Dutch controls.

	m.3243A>G	Healthy	P value
Height men (cm)	178.4 (±6.9)	180.9 (± 7.9)	0.1
Height women (cm)	164.8 (± 8.3)	167.5 (±7.1)	0.004 *
Weight men (kg)	75.8 (±12.8)	84 (±12.8)	0.002 *
Weight women (kg)	65.2 (±17.7)	70 (±12.8)	0.005 *
BMI men (kg/m^2)	23.8 (±3.6)	25.6 (± 5.4)	0.08
BMI women (kg/m^2)	23.9 (±6)	24.6 (± 8.4)	0.5
BMI category			0.00 *
Overweight or obesity: BMI >25 n (%) men	11 (37)	588 (59.9)	
Overweight or obesity: BMI >25 n (%) women	19 (30)	444 (42.2)	
Healthy weight: BMI 20-25 n (%) men	16 (53)	391 (39.9)	
Healthy weight: BMI 20-25 n (%) women	34 (54)	595 (56.5)	
Low BMI < 20 n (%) men	3 (10)	2 (0.2)	
Low BMI < 20 n (%) women	10 (16)	14 (1.3)	

**P=<0.05*

Female carriers were 2.7 cm shorter (average 164.8 cm versus 167.5 in healthy p= 0.004) than healthy controls, in male carriers there was no significant difference between carriers and healthy controls (average 178.4 m versus 180.9 in healthy controls p= 0.099) (Table 1). Male subjects weighed 8.2 kg less than controls (75.8 versus 84 kg p= 0.002) and females weighed 4.8 kg less (65.2 versus 70 kg p= 0.005).

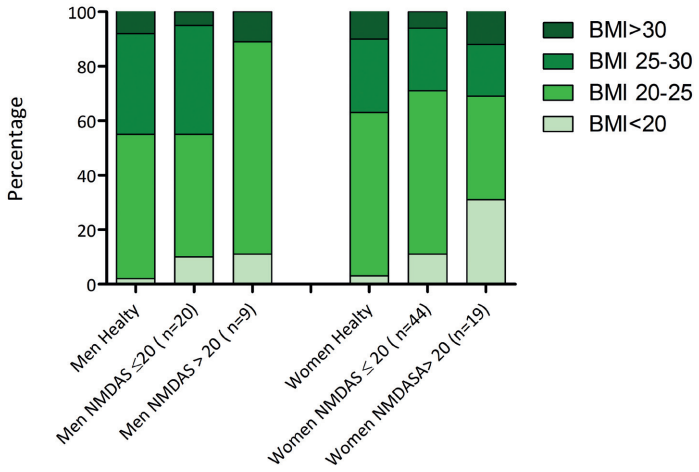

Figure 1. Body mass index in male and female m.3243A>G carriers and healthy Dutch controls.

Carriers had a significantly less frequency of overweight (BMI> 25 kg/m^2) or obesity (BMI>30 kg/m^2) (32%) compared to the average Dutch population (48%) (Figure 1). They were significant more frequently underweight (BMI< 20 kg/m^2) than the average Dutch population: 14% versus 2%. Of the women with a NMDAS score above 20, 31% were underweight. Carriers with BMI <20 kg/m^2 had a significantly higher NMDAS (p=0.01). A negative correlation between BMI and heteroplasmy levels in UEC was present in carriers of the m.3243A>G mutation (spearman c.c.= -0.319, p= 0.003). Nineteen percent (n=17) of the carriers had a MUST score of 1 or 2: average (12%) to high (7%) risk for malnutrition. In 14 carriers this score was based on their low BMI. Of the other 3 carriers with a risk for malnutrition 2 had a healthy weight and one was overweight, there MUST scores were based on more than 5% loss of bodyweight in the last 6 months.

The majority of carriers (81%) had a MUST score of zero: no risk of malnutrition. Five percent of the m.3243A>G carriers used medical feeding supplements; no patient was dependent on tube feeding.

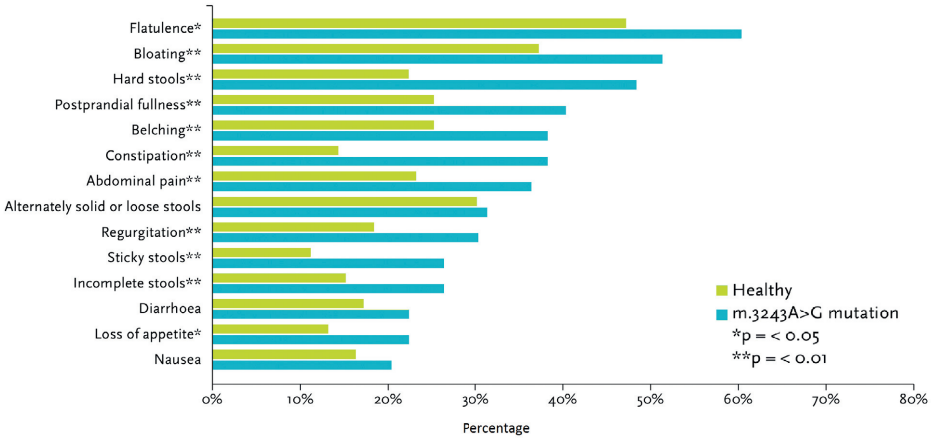

Figure 2. Gastrointestinal symptoms in m.3243A>G carriers and healthy Dutch controls.

Gastrointestinal complaints

In the four weeks before answering the questionnaire, 79 carriers (86%) suffered from at least one gastrointestinal symptom. Nearly all gastrointestinal complaints had a higher frequency and increased severity in carriers of the m.3243A>G mutation compared to the average Dutch population (Figure 2). Most frequent symptoms were bloating, hard stools and flatulence. Nausea and diarrhea and alternately solid or loose stools were also common but there was no significant difference to the controls for these symptoms.

Hard stools or constipation were reported by respectively 48% and 38% of the carriers, whereas controls only reported these complaints in respectively 22% en 14% ($p<0.001$). Fourteen percent of the patients used laxatives and 44% had a stool frequency of less than once a day.

Overall, 69% of the carriers of the m.3243A>G mutation reported one or more constipation-related complaint (use of laxatives, stool frequency of < 1 time a day, hard stools and/or self-reported constipation).

Mean severity score of gastrointestinal problems was 10.7 (± 11.9) range 0-50. Carriers with a BMI <20 ($n = 13$) ($p = 0.028$) and female carriers ($p=0.009$) had a significantly higher severity score of gastrointestinal complaints. Patients with postprandial fullness ($p=0.045$) or vomiting ($p=0.048$), had a significantly lower BMI compared to carriers without this specific symptom. Severity of gastrointestinal problems was not clinically relevant related to BMI (correlation coefficient -0.152, $p= 0.013$)

Gastrointestinal symptoms that were most frequently reported as severe (5, 6 or 7 on the 7-point-likert scale) were; hard stools (14%), constipation (11%), flatulence (11%) and bloating (10%). All these complaints were significantly more frequently scored as severe by carriers compared to controls. Also significantly more frequently scored as severe were regurgitation (8%), post-prandial fullness (9%), belching (10%), dysphagia for solids (2%), incomplete stools (8%) and sticky stool (7%).

One patient in this cohort had a pancreatitis in the past, two patients needed surgery for severe constipation.

There was no correlation between heteroplasmy levels in UEC and gastrointestinal complaints. Frequently reported symptoms in patients carrying the m.3243A>G mutation are myopathy and diabetes. The presence and severity of these symptoms had no significant relation to the gastrointestinal problems.

Dysphagia

In this study 21% of m.3243A>G carriers had trouble swallowing, as scored by the physician who performed the NMDAS. When we specifically asked for all sub-items of the NMDAS swallowing score in the questionnaire this frequency was much higher: 45%. Using the Gastrointestinal Symptoms Questionnaire, dysphagia seems to be a frequent problem (33%), but the severity was not very high (Figure 3). Liquids are less a problem compared to solids, the difference with controls was significant both in the carriers who have trouble with liquids ($p=0.008$) as with solids ($p<0.001$).

No significant differences in BMI were present in carriers reporting dysphagia compared to carriers without dysphagia. In six carriers with more severe complaints of trouble with

swallowing liquids there was a significant difference in BMI: whereas carriers without trouble swallowing liquids had an average BMI of 24.1 (±5.5), carriers with such problems had an average BMI of 20.7 (±3.1) (p=0.046).

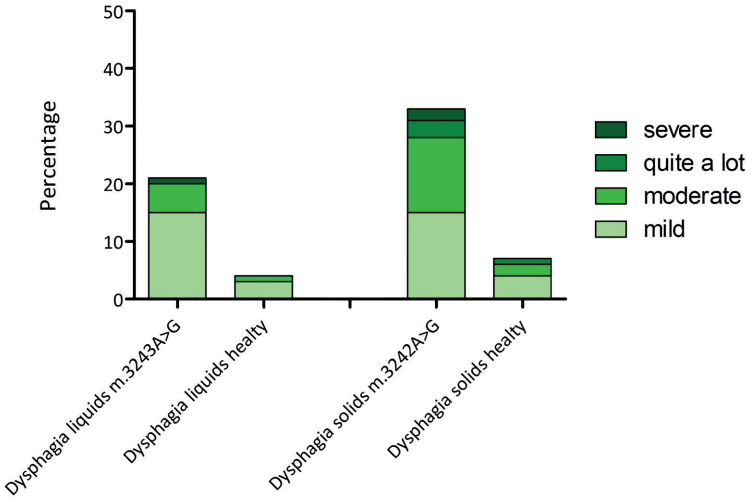

Figure 3. Dysphagia in solids and liquids in m.3243A>G carriers and healthy Dutch controls.

DISCUSSION

As there currently is no cure for mitochondrial disease [32], optimizing nutrition and treating major complaints such as constipation may be one of the few methods to improve quality of life. Because gastrointestinal symptoms are very common in these carriers as well as in the general population, and these often have a non-specific character, there may be a considerable risk for under-diagnosis of these possibly treatable problems.

The present study shows for the first time in a robust cohort that dysphagia and gastrointestinal problems, and more specifically constipation, are very common in carriers of the m.3243A>G mutation. These complaints are common in the total patient cohort and are not related to heteroplasmy levels in UEC, disease severity or the presence of diabetes.

The severity of gastrointestinal problems as well as the overall disease severity and heteroplasmy levels in UEC are associated with a decreased BMI and an increased risk for the development of malnutrition. Strong points of this investigation include the high number of enrolled carriers with a homogeneous genetic background, despite the low incidence of this disease. Also, the high response rate renders these data highly relevant for the guidance and treatment of these carriers.

The high frequency of gastrointestinal problems has been previously described [17, 18, 21]. We compared our cohort of 92 carriers of the m.3243A>G mutation with a cohort of 1627 healthy Dutch controls to identify those gastrointestinal problems that are specifically more frequent in carriers of the m.3243A>G mutation. Our study shows that motility problems of the bowel are probably the most frequent symptom: for instance, constipation was found in 69% of carriers of the m.3243A>G, i.e. by far higher than in healthy controls.

Patients presented with a range of underlying gastrointestinal problems. Some were diagnosed with irritable bowel syndrome (IBS) prior to the mitochondrial diagnose which seems not surprising given the diversity of symptoms. Although nausea, diarrhea and alternately solid or loose stools are common in this cohort, similar symptoms occur just as frequent in the control population and seem not specific for the m.3243A>G mutation, yet remain potentially treatable.

We show in this study that severe gastrointestinal symptoms increase the risk for malnutrition in carriers of the m.3243A>G mutation. Fourteen percent of carriers had a BMI of <20 kg/m^2 and in more severely affected patients (NDMAS >20) even up to 31% had a BMI of <20 kg/m^2. There was no relevant correlation between gastrointestinal problems and BMI, indicating that probably several other confounders such as depression or fatigue may contribute to the risk for malnutrition. Screening for malnutrition or gastrointestinal symptoms can lead to early detection, and hence early treatment of these problems. Since malnutrition has been related to secondary mitochondrial dysfunction [33-35] and may worsen outcomes, like in other disease [36, 37], this remains a major concern in these patients.

Dysphagia may well be more common than both doctors and patients realize. In a non-specific questionnaire for swallowing problems (as included in the NMDAS), a low prevalence of dysphagia was reported. For instance, in our previous study we reported an 18% incidence of dysphagia in a cohort of m.3243A>G carriers [24]. In a partly overlapping cohort we now found that 45% of patients suffered from dysphagia using a more specific questionnaire based on the NMDAS.

A patient-reported incidence of dysphagia of 33% was found using the Gastrointestinal Symptoms Questionnaire. These differences in reported incidences of dysphagia show that the method to diagnose dysphagia is instrumental when assessing its incidence and suggests that the NMDAS underestimates this problem.

Dysphagia usually develops slowly and is not severe in most patients, and patients may therefore adapt their eating pattern sufficiently. This notion might also explain why no differences in BMI between patients with mild dysphagia and without dysphagia were found. As with other neuromuscular disorders, solid foods seem to cause more problems

than liquids [38]. Muscle weakness is the most likely cause for dysphagia in this patient group and demands a different treatment strategy than dysphagia from other causes [19, 38]. For example, in dysphagia due to cerebral damage, it is very common to prescribe thickened drinks which will aggravate dysphagia in mitochondrial patients and will take more energy to consume.

In this study we used low BMI as a marker for malnutrition. Because it is an easily available continue variable suitable for comparison with healthy controls and for statistical analyses. BMI however is not a validated variable for malnutrition in neuromuscular disorders and it is known that patients with normal BMI could suffer from low fat free mass which is also an important marker for malnutrition. In future studies we would like to recommend using body composition as an additional marker.

In conclusion, gastrointestinal problems and dysphagia are common in carriers of the m.3243A>G mutation. The severity of the gastrointestinal problems as well as the overall disease severity is associated with an increased risk for the development of malnutrition. The common disease scores used to identify severity of disease in mitochondrial patients, are insufficient to recognize these gastrointestinal complaints. Healthcare professionals treating patients with the m.3243A>G mutation should be aware of this high prevalence and should therefore actively ask for gastrointestinal problems to ensure a timely treatment of these problems.

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DISCLOSURES

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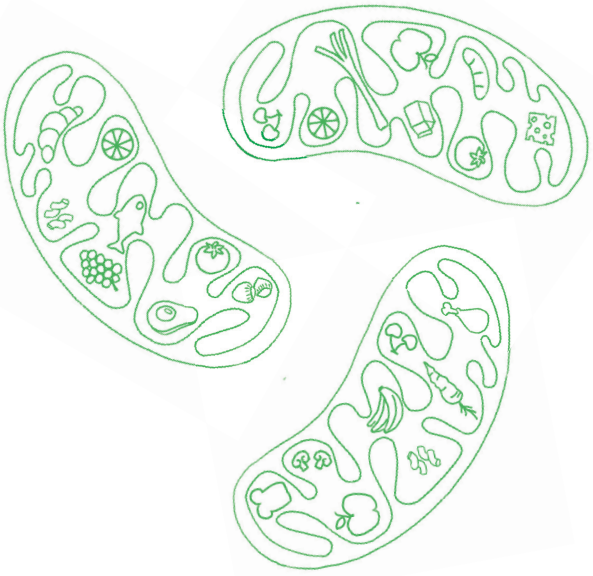

CHAPTER 3

Patients with mitochondrial disease have an inadequate nutritional intake

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ABSTRACT

Background

Mitochondrial disease (MD) is a group of disorders caused by dysfunctional mitochondria, the organelles that generate energy for the cell. Malnutrition in MD patients may lead to increased mitochondrial dysfunction, which may enhance already existing symptoms. The aim of this study was to investigate whether MD patients have an insufficient or unbalanced food intake and to establish which nutrients and product groups are particularly compromised in this patient group.

Methods

In this observational, cross-sectional, retrospective study, sixty 3-day nutrition diaries of adult MD patients were analyzed and compared to the Dutch Recommended Daily Allowance (DRDA) and to the Dutch national Food survey (DnFs).

Results

The intake of all macro- and micronutrients of MD patients was significantly different from DRDA values except for fat and iron. In particular, protein and calcium intake in MD patients was significantly lower compared to DnFs. Inter-individual differences were high. Also, intake of fiber, sugars, saturated fat, and vitamin D differed from recommendations for the overall population. In comparison with DnFs the intake of dairy products and drinks was significant lower in patients.

Conclusions

Our study demonstrates that many patients with MD have an inadequate diet. Specifically, intake of protein, calcium, dairy products and fluids were low. Overall, eating a healthy diet seems as difficult for MD patients as for the general population. Since inter-individual differences are high, individual diet counseling is recommended for all adult MD patients.

Clinical Relevancy Statement

For MD patients using a healthy adequate diet is of utmost importance because of the increased risk for malnutrition, gastrointestinal and metabolic problems, muscle weakness and fatigue. We found that patients with MD have an inadequate diet rendering them at risk for malnutrition. These findings are clinically relevant for clinicians and dieticians, because a cure for MDs is lacking and treatment remains symptomatic. Since malnutrition can be treated, with the promise to improve the quality of life, offering individual diet counseling seems a promising treatment option in adult MD patients.

INTRODUCTION

Mitochondrial disease (MD) is a group of genetic disorders caused by dysfunctional mitochondria: the organelles that generate energy for the cells. Mitochondria are present in every cell of the human body - except red blood cells - and convert the energy of nutrients into ATP which powers most cell functions. Tissues that require major amounts of energy, such as the brain, skeletal muscle and heart, are most commonly and severely affected.

MD is a heterogeneous, multi systemic and progressive disease. The heterogeneity of MD is caused by dualistic genome control by mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). The mitochondrial 3243A>G mutation is the most frequently reported genotype. MD may present from infancy to adulthood. Patients can be strongly affected and may die at childhood or may present with a milder disease phenotype. Maternally Inherited Diabetes and Deafness (MIDD), Mitochondrial Myopathy (MM), and Chronic Progressive External Ophthalmoplegia (CPEO) are the most frequent reported phenotypes in adult patients [1].

MD patients frequently suffer from conditions that may affect nutritional intake and metabolism such as gastrointestinal problems, dysphagia, fatigue, or diabetes. Accordingly, in a previous study in patients with MD caused by the m3243 A>G mutation we showed that 86 percent of patients suffer from one or more chronic gastrointestinal manifestations, mainly constipation, bloating, and flatulence. The severity of the gastrointestinal problems as well as the overall disease severity was associated with a lower BMI [2].

Vice versa, it is known from other diseases such as anorexia [3] or cancer cachexia [4, 5] that malnutrition per se causes secondary mitochondrial dysfunction. From data of children with mitochondrial disorders it was suggested that by improving the nutritional status in MD patients ATP production may be improved [6]. MD cannot be cured, and treatment remains symptomatic with the aim to improve quality of life. However, malnutrition can be treated, with the promise to improve quality of life, as has been shown in other diseases, such as head and neck cancer [7] and COPD [8].

Vitamin B and D, iron and calcium are relevant micronutrients in mitochondrial disorders, because deficiencies in these micronutrients may induce frequently observed complaints in these patients, such as fatigue and muscle pains. Also, these micronutrients play an important role in mitochondrial function [9, 10]. Although micronutrient supplementation has been often discussed as a possible treatment strategy in MD patients, the effects are yet not proven [9, 11] In addition, data on intake in MD patients of these micronutrients are lacking.

MD patients often report that they lack energy to prepare and consume healthy meals. This may lead to an imbalanced diet with high intake of snacks and sugar. This is a concern,

because snacks and sugar have low nutritional value which means that patients with a normal or high BMI may be malnourished because of inadequate protein and/or micronutrient intake.

Before designing a proper dietary intervention for MD patients, the malnutrition problem should be analyzed in detail. Disease related malnutrition according to Stratton [12] is a shortage and/or disbalance of nutrients intake leading to negative changes in body composition, functioning, and clinical outcome. Malnutrition is a complex problem and according to the definition of Stratton: both undernutrition as well as obesity could be a status of present, and both are known to occur in MD patients.

In the present study we focused on the definition of malnutrition [12], i.e. nutrient intake. We investigated whether MD patients have an insufficient and/or unbalanced food intake. Furthermore, we analyzed which food groups and nutrients are particularly compromised in this patient group. The intake of the MD patients was compared not only with the recommended daily allowances but also with the Dutch national Food survey [13].

MATERIALS AND METHODS

In this observational, cross-sectional retrospective study, nutrition diaries of eighty adult MD patients of > 18 years of age were collected between the start of 2013 and the end of 2016. All patients were treated at the national expertise center for mitochondrial disorders: Radboud Center for Mitochondrial Medicine (www.RCMM.info). Nutrition diaries were included from patients who gave written informed consent. Patients were excluded when the MD was not genetically proven and when food diaries were not completed for a minimum of three days. The study was approved by the medical Ethics Committee of the Arnhem and Nijmegen region.

Patients completed the nutrition diaries after reading written instructions from a preprinted form. They were instructed to register very accurately all foods and drinks that were consumed during three days, preferably two weekdays and one weekend day. Patients were asked for clarification of their nutritional intake in case the diary was not sufficiently clear. In unresolved cases portion sizes were estimated using the Dutch food measurements codebook [14]. Calculation procedures including the estimations were documented in a log for consistency.

Anthropometrics were measured at the outpatient clinic. Height was measured in a standing position with a calibrated ruler in cm (Seca®). Weight was measured in kilograms on a calibrated electronic scale (Seca®). Information about genotype, phenotype, and presence of diabetics was collected from the electronic patient files.

The nutrition diaries were analyzed according to a standardized procedure. Nutritional intake was calculated twice by two independent research dieticians using FoodFigures® software based on the Dutch food file (NEVO®) 2013 database. No differences were observed between both analyses.

FoodFigures® analyses were made separately on a nutrient and on a food product group level. Total daily protein, fat, saturated fat, and carbohydrate were calculated (g/day). Relevant micronutrients [9] were calculated only if they could be reliably obtained and calculated based on a nutrition diary. This was the case for vitamin D, calcium, vitamin B12, and iron. The calculations were solely based on the nutrition diaries, including enteral nutrition but without the micronutrient supplements. The micronutrient supplements were being checked and reported separately.

To determine if the food intake of patients was sufficient it was compared with various references. The energy intake was compared to the patient's individual estimated requirement. The energy requirement was calculated using the Harris and Benedict equation [15] with a standardized low activity factor of 40%, based on previous actometre measurements in this patient population [16]. Protein intake was compared with the standard recommendation for healthy adults of 0.8 g/kg. For protein we calculated the requirements with body mass, and a correction for weight was applied in patients with a BMI outside the healthy range. Weight was corrected to a BMI of 20 kg/m^2 for patients with a BMI below 20 kg/m^2. For a BMI > 30 kg/m^2, weight was corrected to a BMI of 27.5 kg/m^2 [17]. The fiber intake was compared to the Dutch adult fiber guidelines of 3.4 grams fiber/mega joule. The intake of carbohydrate and fat was compared to standards of the Health Council of the Netherlands (carbohydrate 40 energy% and fat 20-40 energy%). For the micronutrients and product groups, the Dutch Recommended Daily Allowance (DRDA) was used as reference. Finally, the intake of nutrients and product groups was compared to the Dutch national Food survey (DnFs) [13]. Because references differed with age and sex, absolute numbers were converted into percentages of the reference values in which 100% equals the recommendation. For fat the upper limit of the healthy range, i.e. 40 energy%, was set equal to 100%.

Descriptive statistics were used to present the nutrient intake. Data were checked for normality and means were presented with their standard deviation or medians with their respective range. In case of normal distribution, one sample T-test was used to calculate the significance of the difference between the mean intake of the MD patients and the reference data. Correction for multiple testing was performed using the Bonferroni method and only results with a $p < 0.0025$ were considered significant. Pearson's correlation analysis was performed between food groups and relevant nutrients that significantly differed from the DnFs.

RESULTS

Research Population

Eighty nutrition diaries were collected from the Dutch adult MD patients population of the RCMM. Twenty reports were excluded, see Figure 1. The study population available for data analysis consisted of 60 nutrition diaries of 60 MD patients.

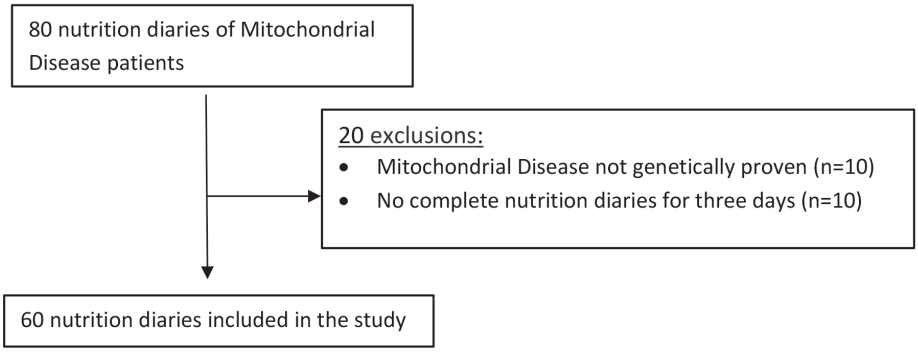

Figure 1. Patient report exclusion schedule.

Patients characteristics

Table 1 shows the characteristics of the MD study population. Nutrition diaries of nineteen males (32%) and 41 women (68%), with a mean age of 49.2 years (± 13.1) and a mean BMI of 24.3 kg/m^2 (±4.6) were included in the study. The most frequent MD phenotypes were Mitochondrial Myopathy (MM, 35%), Maternally Inherited Diabetes and Deafness (MIDD, 30%) and Chronic Progressive External Ophthalmoplegia (CPEO, 30%). Thirty-tree percent of patients suffered from diabetes of which 80% were insulin dependent.

Table 1. Patients characteristics.

Phenotype n (%)	Gender men n (%)	Age (years) Median (range)	Height (cm) Median (range)	Weight (kg) Median (range)	BMI[f] (kg) Median (range)	Diabetic n (%)
MIDD[a] 18 (30)	1 (6)	51 (33-68)	164 (149-176)	57 (39-97)	21.4 (17.7-35.2)	18 (100)
CPEO[b] 18 (30)	13 (72)	59 (42-72)	177 (162-193)	81 (60-144)	26.6 (20.5-41.7)	2 (15)
MM[c] 21 (35)	4 (19)	49 (21-66)	166 (156-185)	67 (48-84)	22.6 (16.7-28.8)	0
Other[d] 3 (5)	1 (33)	33 (30-43)	170 (163-189)	64 (59-83)	23.2 (20.4-24.0)	0
Total 60 (100)	19 (32)	50 (21-72)	169 (149-193)	69 (39-144)	23.6 (16.7-41.7)[e]	20 (33)

a) MIDD= Maternally Inherited Diabetes and Deafness caused by m.3243A>G mutation.

b) CPEO= Chronic Progressive External Ophthalmoplegia including one patient with Kearns–Sayre syndrome. Caused by nuclear POLG (n=6) or twinkle (n=1) mutation or multiple mtDNA deletions (n=7) or mtDNA point mutations (n=4).

c) MM= Mitochondrial Myopathy in 20 patients caused by the m.3243A>G mutation (phenotype other than the classic MIDD or MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes) phenotype. In one patient caused by another mtDNA point mutation.

d) MERFF: Myoclonic Epilepsy with Ragged Red Fibers (n=1) LOHN: Leber's Hereditary Optic Neuropathy (n=1) and Leigh-like disease (n=1).

e) BMI< 20: n=6 (10%) BMI 20-25: n=32 (53%) BMI> 25: n=22 (37%).

f) BMI= Body Mass Index (kg/m^2).

Macronutrients and micronutrients

Figure 2 shows the mean intake of micro- and macronutrients in relation to reference values with corresponding standard deviations, compared to the average intake of the Dutch national Food survey (DnFs).The mean macro- and micronutrient intake of the MD patients was different from reference values ($p<0.0025$) with the exception of fat and iron. While the intakes of energy, fiber, vitamin D, and calcium were lower than the reference data, the intakes of carbohydrate, protein, saturated fat, and vitamin B12 were higher than recommendations. Variation in nutritional intake was high, especially in vitamin B12.

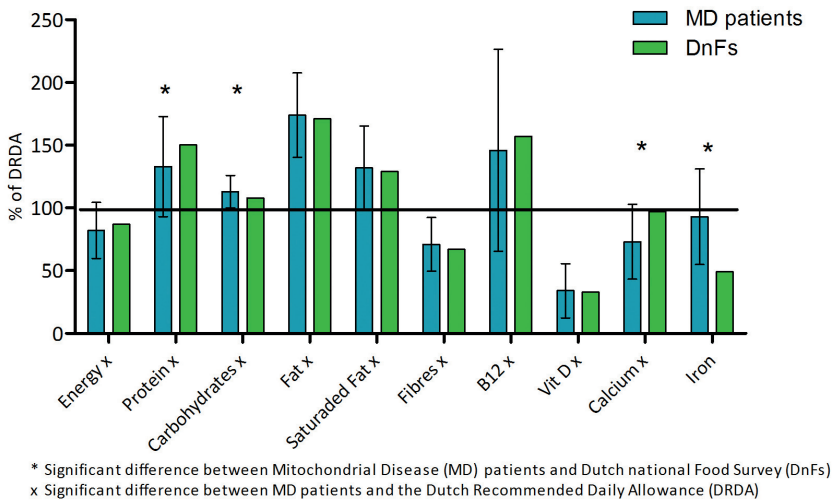

Figure 2. Intake of macro- and micronutrients in MD patients and DnFs expressed as percentage from DRDA. (Mean ± SD)

Protein intake was higher than the Dutch Recommended Daily Allowance of 0.8 g/kg. The mean protein intake in the MD patients was 1.1 g/kg (SD ± 0.34).

Comparing the patients' intakes to the DnFs, the intake of protein and calcium was significantly lower in MD patients. In contrast, the intake of carbohydrates and iron was significantly higher in this group.

Product groups

The intake of food per product groups of the MD patients was significantly lower than the reference values ($p< 0.0025$), with the exception of the product group 'cheese' (Figure 3). The intake of food for each product groups was below the DRDA, while the intake of meat was higher than the DRDA.

The intake of 'dairy products' and 'drinks' was significant lower in the MD patients compared with the DnFs. Furthermore, although below DRDA reference, the intake

of food from the 'bread and potatoes' group was significantly higher in MD patients compared to the Dutch national Food survey.

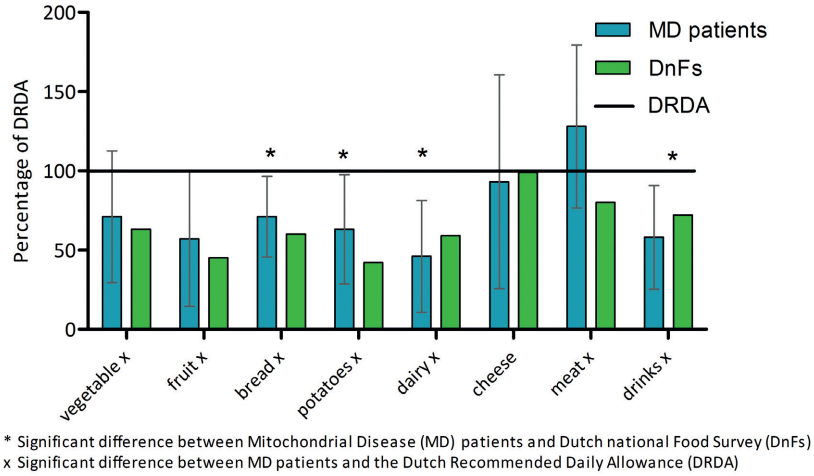

Figure 3. Food group intake in MD patients and DnFs expressed as percentage from DRDA (Mean ± SD)

Correlation dairy products with protein and calcium

A correlation of 0.74 ($p<0.001$) was observed between the consumption of calcium and dairy products. Furthermore, a correlation of 0.4 ($p= 0.002$) was observed between the consumption of protein and dairy products.

Snacks, soft drinks, and mono- and disaccharides

Patients consumed 72 g (± 56.5 g) of snacks a day, which is not significantly different from the average Dutch intake. The mean intake of soft drinks was higher in the patients while the intake of sugars was significantly lower than the average Dutch intake, but still above recommendations according to DRDA. When categorizing the MD patients in diabetic or non-diabetic patients this lower intake of mono- and disaccharides was only found in the diabetic group (n=20) (Table 2).

Table 2. Mean intake of snacks, soft drinks, and mono- and disaccharides in MD patients.

	Mean MD patients (±SD)	Mean DNFCS
Snacks (g)	72 (±56.5)	63
Softdrinks (ml)*	249 (±421.5)	86
Mono- and disaccharides (g)*	86.5 (±42.6)	102
Non-diabetic patients	93.5 (±47.1)	
Diabetic patients *	72.6 (±28.1)	

*DNFCS, Dutch National Food Consumption Survey; MD, mitochondrial disease. *significance difference with Dutch national Food survey, T-test, P<0.0025*

Supplement use

Information on nutritional supplement use of 44 patients was obtained. Of these, 68 percent consumed one or more nutritional supplements. Coenzyme Q10 was mostly used (44%) followed by multivitamin supplements (32%). One patient used enteral nutrition.

DISCUSSION

The aim of this study was to describe which nutrients and/or product groups are specifically different from reference values in adult MD patients. The main finding was that the nutritional intake of adult MD patients is inadequate according to the DRDA and the individual variation in intake is high. However, this also applies to the healthy Dutch population. Eating adequately seems to be difficult for everyone. It is particularly important, however, for MD patients since an insufficient diet may increase the risk for malnutrition, leading to secondary mitochondrial dysfunction, resulting in worsening of symptoms [6].

Importantly, the average protein intake of MD patients was significantly lower compared to the healthy Dutch population, but still well above the recommendations for healthy individuals. As MD patients are not healthy, protein requirement may be different in these individuals. For instance, in some patients with renal involvement the protein intake should be restricted [18]. On the other hand, malnutrition is frequently found, leading to increased protein needs. Protein recommendations in malnutrition are minimal 1.2 g/kg [19] which is higher than the mean intake of the MD patients. This lower protein intake could be partly explained by the very low intake of dairy products which is specific for the MD patients: a moderate correlation between protein and dairy products was calculated. Avoidance of dairy products may be explained by frequent gastro-intestinal problems, especially related to bloating and abdominal cramps [2]. Adequate intake of dairy products is important in MD patients not only because of protein, but also because of calcium requirements. Correlation between dairy products and calcium intake was strong; calcium intake was too low in MD patients, increasing the risk for osteoporosis [20]. MD patients already have a higher risk for osteoporosis because of low physical activity and persistent lactic acidosis [21]. In addition, vitamin D intake was, although not different between MD patients and DnFs, extremely low. An adequate vitamin D status is particularly important in MD since, in addition to the risk of osteoporosis, vitamin D deficiency may cause muscle weakness [22] which is a key complaint in these patients [1]. Taken together the inadequate intake of protein and dairy products is a serious concern in the diet of MD patients.

Other relevant findings of this study were that MD patients tend to have lower fluid intake than the DnFs and a to lower dietary fiber intake compared to the recommendations. As fluid en fiber intake is important in preventing and treating the frequently occurring gastrointestinal distress in these patients [2] caution should be paid to these nutrients.

In our study, the energy intake was insufficient, but not different from the DnFs. It is known that the energy demands of MD patients are usually below recommendations for healthy individuals because of the lower physical activity [16]. Because it is assumed that their nutritional requirements are not different [9] from normal individuals, the nutrient density of the diet should therefore be higher than in patients with normal energy demands. However, we did not find this compensating eating pattern in the MD patients. Data from our study did not support our hypothesis that MD patients eat more unhealthy than the general population. Although they did consume high levels of sugar, snacks, and saturated fat, this was not different from the consumption of the general Dutch population. Nevertheless, healthy eating is particularly important for MD patients, because of the increased risk for diabetes, fatigue, and metabolic syndrome [1, 23]. The high saturated fat intake is relevant, as cholesterol-lowering statins is discouraged in MD patients because of the risk of statin myopathy [24].

Previous research regarding supplement use in MD patients in a partly overlapping cohort (n=33) revealed that 70% of patients used nutritional supplements [25]. These data are corroborated by our findings in this cohort (68%). The most commonly used products were coenzyme Q10 and multivitamin supplements. The supplement use is not in line with the identified deficiencies in the diet because calcium, vitamin D, fiber or protein supplements were barely used. Given the increased incidence of malnutrition in MD patients [2] prescribing enteral nutrition or mineral and vitamin supplements tailored according to the patient's individual deficiencies should be considered.

A strong point of this study is the relative large group of subjects with this rare disorder. Although the patient population was heterogeneous, this study provides unique insight in the food intake of MD patients in general.

A weakness of the study is that the use of nutrition diaries may lead to bias in the results. Interpretation errors, as well as underreporting or overreporting, are possible reasons for this [26]. However, in a retrospective study it is not possible to change the method of data collection, and nutrition diaries provide valuable information that qualitatively seems better than the frequently used dietary history [27]. Furthermore, the data collection method used in the DnFs is the 24-hour dietary recall of any two nonconsecutive days. This method is different from the method used in our study. These weaknesses should be taken into account when interpreting the study results.

Overall, it may be concluded that patients with MD have an inadequate diet. Calcium, dairy products, and fluid intake are often decreased. Furthermore, intake of fiber, sugars, saturated fat, and vitamin D intake differ from general recommendations. Using a healthy and adequate diet is especially important in MD patients, because of the increased risk of gastrointestinal problems, diabetes, metabolic syndrome, muscle weakness, fatigue, and osteoporosis. Because individual differences are considerable, a patient-tailored

approach is recommended in which all adult MD patients are provided with individual diet counseling for obtaining a healthy adequate diet.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to report and no funding was given for this study.

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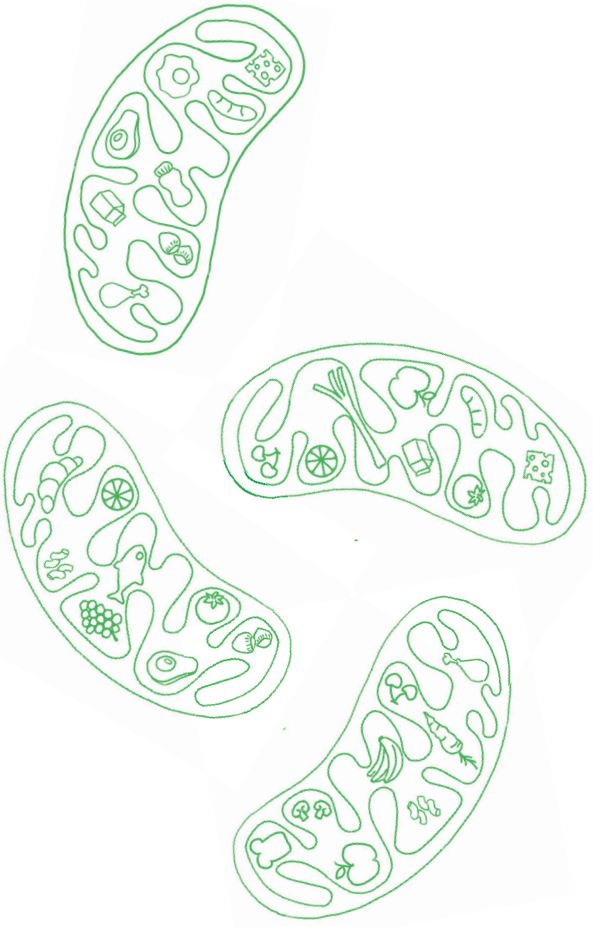

CHAPTER 4

Association of body composition, physical functioning and protein intake in adult patients with mitochondrial diseases. DYNAMO study

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The Acronym DYNAMO stands for DYnamic (physical functioning) and Nutritional Assessment in MitOchondrial diseases

ABSTRACT

Background

Whether decreased physical functioning of patients with mitochondrial disease (MD) is related to altered body composition or low protein intake needs clarification at the background of the nutritional state.

Methods

In this 2-site cross-sectional study, MD patients were age-, BMI- and gender-matched to controls. Body composition was assessed by dual-energy x-ray absorptiometry. Physical functioning was measured by handgrip strength, 6-minute walking test (6MWT), 30-second sit-to-stand test (30SCT), and 6-minute mastication test (6MMT). Total daily protein intake was calculated by three-day food records. Malnutrition was assessed by Patient-Generated Subjective Global Assessment and the Global Leadership Initiative on Malnutrition (GLIM) criteria, and sarcopenia by the 2018 consensus. Data were analyzed using independent samples *t*-tests, Fisher's exact test and Spearman and Pearson's correlation coefficients.

Results

Thirty-seven MD patients (42±12 years, BMI: 23±4 kg/m^2, 59% female) and 37 matched controls were included. Handgrip strength was moderate inversely related to fat mass index in both MD patients and controls, whereas it correlated with fat free mass index in controls solely. Protein intake was associated with muscle strength (handgrip strength and 30SCT) in MD patients but not in controls. Twenty-seven MD patients (73%) were malnourished and five (14%) were classified as sarcopenic.

Conclusions

Muscle strength is related to body composition and protein intake in MD patients. This in combination with the high incidence of both malnutrition and sarcopenia warrants individual nutritional assessment in MD patients.

Clinical Relevancy Statement

Physical functioning is chronically impaired in adult MD patients implicating decreased quality of life. Insights in the relation between protein intake, body composition and physical functioning in these patients and the prevalence of malnutrition and sarcopenia could help to improve patient care.

INTRODUCTION

Approximately one out of 5000 persons worldwide is confronted with mitochondrial disease (MD) [1], a genetic neuromuscular chronic disorder causing intracellular energy (ATP) shortage [1]. As MD patients experience a wide range of complaints, such as fatigue, muscular weakness, gastrointestinal complaints, dysphagia and exercise intolerance [2, 3] treatment focuses on symptom relief, securing physical functioning and quality of life [2].

Poor physical functioning is predicted by a body weight that is either too low or too high [4-7], conditions that are frequently observed in MD patients [8-12]. Also, body composition is known to be an important factor in physical functioning in the general population as well as in neuromuscular disorders in particular [6, 7, 13, 14]. Decreased physical functioning was related to an increased fat mass in patients with myotonic dystrophy [13] or with decreased muscle mass in patients with muscular dystrophy [7, 15, 16]. In patients with chronic symptoms similar to mitochondrial disease, like kidney disease [17], lower fat free mass and decreased muscle strength were associated. An increased muscle mass and strength may also be related to lower diabetes risk [18]. Altered body composition in MD patients has been reported [9, 12] and higher skeletal muscle mass index in these patients was correlated with higher muscle strength [12]. However, whether physical functioning is related to fat mass has not been established yet.

It is known that physical activity improves physical function and body composition [19]. The decreased physical activity in MD patients [10] is likely to play a role in body composition and physical functioning in these patients. However, it is not known whether MD patients' physical functioning is decreased due to altered body composition and/or if this possibly is influenced by nutritional intake. It is known that physical function in MD patients can be improved by physical exercise [20] therefore exercise is part of standard care in MD patients [21].

The combination of low muscle mass and low muscle strength seen in MD patients may be defined as sarcopenia [22, 23]. Sarcopenia significantly reduces functioning, as well as survival [22, 24]. Therefore, it is relevant to know whether sarcopenia is present in MD patients, in order to adjust treatment accordingly [25].

The low physical functioning in MD patients may also be related to alterations in nutritional intake and the nutritional status. Recent observational studies suggested that MD patients have inadequate protein intake and are at risk of malnutrition [9, 25-27]. This could affect both body composition and physical functioning. Diagnosing malnutrition in MD patients is challenging [9, 28]. Standard screening tools aimed at screening acute malnutrition are not applicable in MD patients as these patients suffer from chronic malnutrition [25]. Recent literature advises to perform nutritional

assessment for determine nutritional state in MD patients [9, 25, 27], but it is not known which measurements are valid nor which cutoff values should be applied.

The primary goal of this study is to explore the association between physical functioning, protein intake and body composition in adult MD patients. Additionally, the prevalence of malnutrition and sarcopenia in this MD population is assessed using various nutritional assessment tools. Finally, the diagnostic accuracy of BIA to determine body composition in MD patients was tested.

METHODS

In this two-site, cross-sectional study the associations between physical functioning, protein intake and body composition were examined in MD patients compared to age-, body mass index (BMI)-, and gender-matched controls. The study protocol was approved by the ethics committee of the Nijmegen-Arnhem region (NL58262.091.16/2016-2667).

Primary and secondary endpoints

The primary endpoint was the association between physical functioning, protein intake and body composition in MD patients. The secondary endpoint is the prevalence of malnutrition and sarcopenia in MD patients.

Study population

Genetically confirmed MD patients and healthy controls were included if they (1) were ≥18 years of age and (2) had signed informed consent. Controls were matched with included MD patients for age- (+/- 5 years), BMI- (+/- 2 kg/m^2), and gender. Exclusion criteria were (1) a pacemaker or implant, (2) pregnancy or lactating, (3) disordered hydration status (edema, dehydration), (4) diagnosed with a (chronic) disease interfering with the nutritional assessment, or (5) acute illness and fever. Additionally, MD patients were excluded in case of (1) non-genetically confirmed MD diagnosis, (2) diagnosis of none myopathic MD phenotype, or (3) if DXA and handgrip strength data were not available.

A sample size of 37 MD patients and 37 controls was calculated using data of MD patients (n=24) and compared with the reference values of Dodds [29] to show a difference of physical functioning between MD patients and controls with a power of 0.80 and an alpha of 0.05 based on an estimated dropout rate of 30% using the handgrip strength as primary outcome variable for physical functioning.

Data collection

MD patients' data were prospectively obtained from the Radboudumc's MD expertise centre (Radboud Centre for Mitochondrial Medicine, RCMM) from March 2015 until

January 2017. MD patients were measured during a four-day multidisciplinary evaluation program (as usual care) on the internal medicine ward. Controls were measured at the Nutritional Assessment Lab of the HAN University of Applied Sciences between August 2017 and July 2018.

Demographic and disease characteristics

Age (years) and gender were collected from electronic records in MD patients or obtained at day of measurement in controls. In addition, MD patients' genotype and phenotype as well as the presence of dysphagia or gastrointestinal problems were registered.

Physical functioning

Physical functioning was assessed according to the applicable standard operating procedures using four tools relevant for MD patients. Muscle strength was measured by handgrip strength (kg) [22, 30] and 30-second sit-to-stand test (30SCT; number of sit-to-stands) [31]. Endurance was measured by the 6-minute walk test (6MWT; meters) [22, 32] and the 6-minute mastication test (6MMT; number of chew cycles) [33].

Anthropometry and body composition

Height (cm), weight (kg), and waist circumference (cm) were measured to the nearest one decimal point. Total fat mass (kg), total appendicular skeletal muscle mass (kg), and regional lean tissue mass (kg) were determined by whole-body DXA (Radboudumc: Hologic®, model Discovery® A S/N 85606; HAN: Hologic®, model Horizon® W S/N 200103) [34]. Fat mass, appendicular skeletal muscle mass, as well as regional lean tissue mass were normalized by dividing total mass (kg) by height (m) squared into fat mass index (kg/m^2), skeletal muscle index (kg/m^2), and regional lean tissue mass index (kg/m^2), respectively.

Additionally, bioelectrical impedance analysis (BIA) measurements were performed according to clinical practice (Bodystat MDD 1500, 50 Hz). Fat free mass (kg) was calculated twice using 1) Kyle formula [35], having the smallest standard error and highest R squared [35] and 2) Dey being validated in elderly [36].

The diagnostic accuracy of the BIA was tested using the DXA as golden standard. The sensitivity and specificity were determined using the cutoff point of <15 kg/m^2 and <17 kg/m^2 for fat free mass index in women and men respectively [37] and obesity as fat percentage >30% for women and >25% for men [38].

Nutritional intake

Nutritional intake was assessed by either a three-day food record or, in case missing in clinic, a dietary history using the nutrition calculation program Madows®. Mean energy (kcal/day) and protein (gram/kg body weight/day) intake were calculated. Number of persons on a specific diet were registered.

Malnutrition and sarcopenia

Malnutrition was assessed by Patient-Generated Subjective Global Assessment (PG-SGA©) (score of ≥ 4 was interpreted as risk for malnutrition and ≥ 9 as malnourished) [39] and the GLIM criteria [30]. Sarcopenia was classified using the handgrip strength. The cutoff point for 70-year-old [22] people as well as the actual age cutoff point for low handgrip strength [29] were applied, as the majority of MD patients is younger than 70 year. Sarcopenic obesity was diagnosed according to Baumgartner [40].

Data management

Data were entered encoded in an online case report form (Castor ©, CIWIT B.V., Amsterdam) and double-checked visually by two researchers.

Statistical analysis

Data were reported as means ± standard deviation (SD), median and interquartile range, or frequencies and percentage of the group or total population, if applicable. Normal distribution of the variables was assessed by Shapiro-Wilk tests. Differences between MD patients and controls were tested using the independent t-test or Mann-Whitney U tests for continuous variables and Pearson chi-square test or Fisher's exact test for categorical variables.

Pearson or Spearman correlation coefficients were determined to assess the relations between physical functioning, (regional) body composition and protein intake. Since fat free mass index and skeletal muscle mass index were associated, only fat free mass index was used in association analysis. Statistical analyses were performed using SPSS statistics (IBM Statistics 23). To correct for multiple testing p-values ≤ 0.02 were considered statistically significant for all analyses.

RESULTS

Thirty-seven MD patients and 37 matched controls (all Caucasian) were included in this study (Figure 1). Mean age was 42±12 years and 59% was female (Table 1). Despite having similar BMI, MD patients had a shorter statue compared with controls. Compared with controls, MD patients were on a (diabetes) diet, experienced gastrointestinal problems or dysphagia more often. The majority of the MD patients were diagnosed with the m.3243A>G mutation (78%) (Table 2).

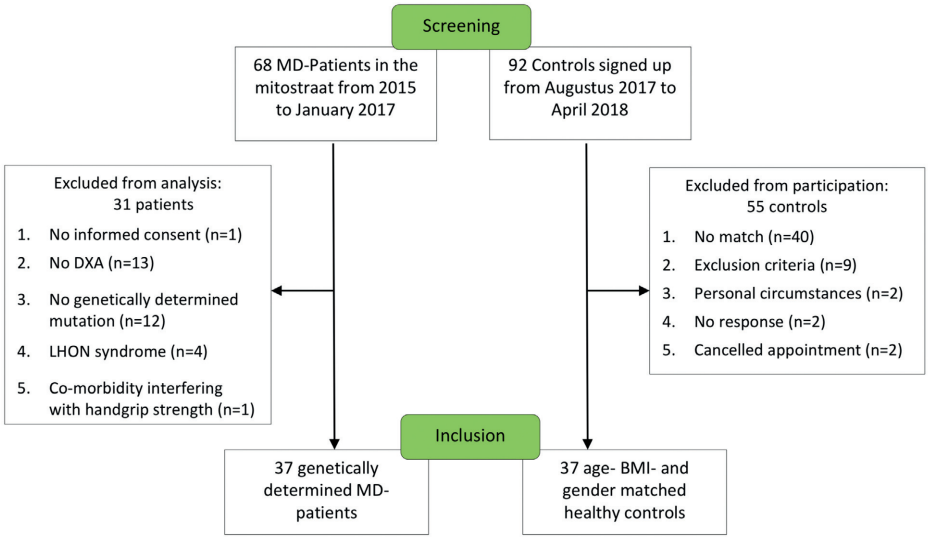

Figure 1. Screening-Flowchart of MD patients and controls in the DYNAMO study.

Table 1. Demographics of MD patients and controls.

	MD patients (n=37)	controls (n=37)	*p*-value
Age (years, mean ± SD)	42 ± 13	42 ± 12	0.84
Female (n/%)	22 (59%)	22 (59%)	
BMI (kg/m^2, median: IQR)	21.9: 19.9 – 24.5	23.0: 21.3 – 25.0	0.54
BMI categories (n/%):			
< 18. kg/m2	2 (5%)	0 (0%)	
< 20 kg/m^2	9 (24%)	4 (11%)	
20-25 kg/m^2	20 (54%)	23 (62%)	
25-30 kg/m^2	6 (16%)	10 (27%)	
>30 kg/m^2	2 (5%)	0 (0%)	
Weight (kg, mean ±SD)	66.6 ± 12.3	72.2 ± 8.9	0.04
Weight loss[1] (n/%)	4 (11%)	2 (5%)	0.39
Height (cm, mean ±SD)	170.8 ± 1.0	176.4 ± 0.9	**0.01**
Waist circumference (cm mean ± SD)	84.3 ± 11.5	81.8 ± 8.0	0.30
High Waist circumference [2] (n/%)	14 (38%)	12 (32%)	0.46
Diet (n/%)	25 (68%)	2 (5%)	**<0.001**
Gastrointestinal problems (n/%)	28 (76%)	5 (13.5%)	**<0.001**
Dysphagia (n/%)	18 (49%)	0 (0%)	**<0.001**

*SD, standard deviation; IQR: interquartile range; P-values ≤0.02 (**in bold**) were considered significant.*
1) Weight Loss according to GLIM criteria: >5% within past 6 months, or >10% beyond 6 months [30]
2) High waist circumference >94 cm (women) and >80cm (men) according to WHO 2008 [44].

Table 2. Disease Characteristics of Mitochondrial Disease Patients (n =37).

	Frequency (n)	Proportion (%)
Genotype		
m.3243A>G	29	78
point mutations (mtDNA)	5	14
nDNA mutation	2	5
deletion mtDNA	1	3
Phenotype		
Mitochondrial myopathy	14	38
MIDD	13	35
MELAS	4	11
CPEO	3	8
Leigh syndrome	2	5
MERRF	1	3

CPEO, Chronic progressive external ophthalmoplegia; mtDNA, mitochondrial DNA; MELAS, Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, Myoclonus epilepsy with ragged-red fibres, MIDD, Maternally inherited diabetes and deafness; nDNA, nuclear DNA

Physical functioning

Physical functioning in MD patients was lower compared to controls based on all physical functioning tests (Table 3).

Body composition

Total appendicular skeletal muscle mass was lower in MD patients compared with controls ($p<0.02$) (Table 3), whereas no differences in total body fat or muscle mass were observed. Also, leg lean tissue mass index was lower in MD patients compared to controls (Table 3).

Association between physical functioning and body composition

Handgrip strength was increased with higher fat free mass index in controls (Pearson $r = 0.67$, $p < 0.001$), but not in MD patients (Pearson $r = 0.30$, $p = 0.08$) (Figure 2a). In all MD patients together no significant association between handgrip strength and skeletal muscle mass index was observed, however a significant association for the m.3243A>G mutation genotype subgroup (Spearman $r = 0.31$ $p = 0.06$ *and* $r = 0.44$, $p < 0.02$ respectively) was observed (Supplement 1). Handgrip strength declined in both MD patients and controls with higher fat mass index (Spearman $r = -0.61$ *and* $r = -0.49$, respectively; $p < 0.01$) (Figure 2b).

Nutritional intake

Energy intake was significantly lower in MD patients compared to controls (Table 3). Moreover, significantly more patients had an energy and protein intake below the recommended intake (Table 3).

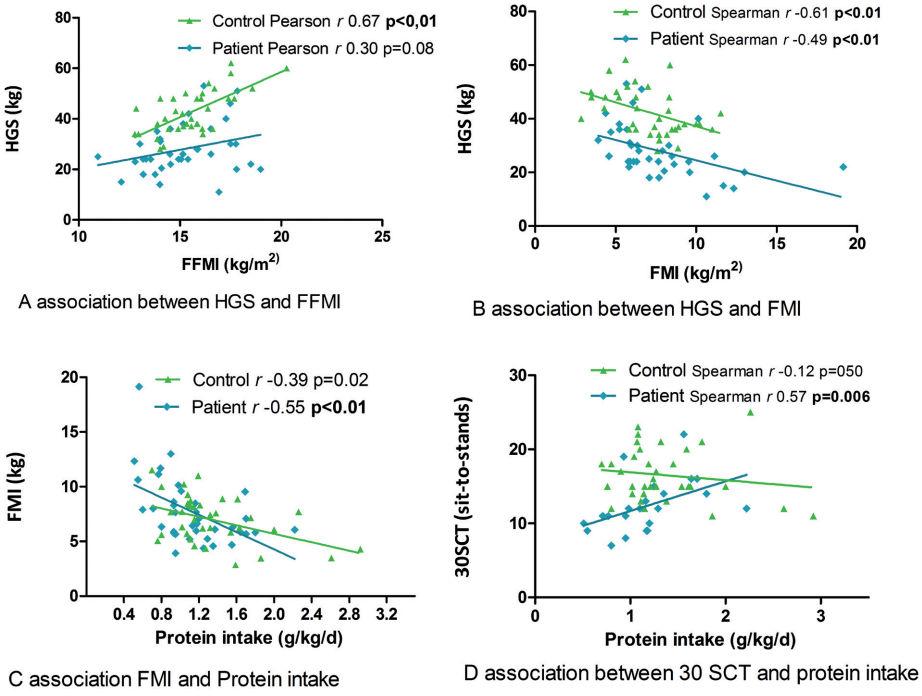

Figure 2. Association between handgrip strength, 30-second sit-to-stand test, fat mass index, fat free mass index and protein intake. HGS, Handgrip strength, FMI, fat mass index, FFMI, fat free mass index, 30SCT, 30-second sit-to-stand test. *P*-values <0.02 **(in bold)** were considered significant

Table 3. Physical functioning, body composition, nutritional intake, malnutrition and sarcopenia in MD patients and controls.

	MD patients (n=37)	controls (n=37)	*P*-value*
Physical functioning test			
Handgrip strength (kg) (mean ± SD)	28 ± 10	43 ± 9	**< 0.001**
Too low handgrip strength [1] (n/%)	6 (16)	0 (0)	
Too low handgrip strength [2] (n/%)	15 (41)	0 (0)	
6-MMT (n chewing cycles) (M ± SD)	396 ± 130 (n=29)	577 ± 141 (n=36)	**< 0.001**
30SCT (n sit-to-stands) (mean ± SD)	12 ± 4 (n=22)	17 ± 4 (n=36)	**< 0.001**
6-MWT (distance in m) (median; IQR)	441: 426-427 (n=20)	681: 635-639	**< 0.001**
6-MWT < 400 m (n/%)	3 (15)	0 (0)	
Total body composition			
FMI (kg/m^2) (median; IQR)	7.7 :6.7-8.7	7.0 :6.3-7.7	0.25
Fat percentage (%) (mean ± SD)	22 ± 7	16 ± 7	0.21
high fat percentage[3] (n/%)	9 (24)	7 (19)	0.21
ASM (kg) (mean ± SD)	17.6 ± 4.0	19.8 ± 3.8	**0.02**
FFMI (kg/m^2) (mean ± SD)	15.2 ± 1.9	15.5 ± 1.6	0.38
SMI (kg/m^2) (M ± SD)	6.0 ± 1.0	6.3 ± 0.9	0.11
Too low SMI[4] (n/%)	25 (68)	21 (57)	0.34
Bone density (g/cm^2, mean ± SD)	0.31 ± 0.83	0.27 ± 0.80	0.99
Osteopenia[5] (n/%)	3 (8)	0 (0)	0.08
Regional LTMI (kg/m^2, mean ± SD)			
Average arm	1.3 ± 0.30	1.3 ± 0.3	0.42
Trunk	12.7 ± 2.0	13.1 ± 1.5	0.34
Average leg	3.8 ± 0.7	4.3 ± 0.6	**0.004**
Nutritional Intake			
Protein intake (g/kg/day) (median; IQR)	1.1: 0.9-1.4	1.2: 1.1-1.7	0.07
Too low protein intake[6] (n/%)	25 (68%)	4 (12%)	**< 0.01**
Energy (kcal/d)	1663 ± 500	2322 ± 644	**< 0.001**
Kcal intake (% of calculated needs, mean ± SD)	81% ± 238	98% ± 251	0.03
Too low Kcal intake[7] (n/%)	25 (68%)	17 (46%)	
PG-SGA[8]			**< 0.001**
PG-SGA[8] 0-4 (n/%)	5 (14%)	35 (95%)	
PG-SGA[8] 4-9 (n/%)	16 (43%)	1 (3%)	
PG-SGA[8] ≥9 (n/%)	16 (43%)	1 (3%)	
Malnutrition[9] (n/%)	17 (46%)	10 (27%)	0.09
Severe Malnutrition[9] (n/%)	1 (3%)	0 (0%)	
Sarcopenia[10] (n/%)	5 (14%)	0 (0%)	**0.02**
Sacopenic obesity[11] (n/%)	4 (11%)	3 (8%)	0.7

*SD, standard deviation; IQR, interquartile range; 30SCT, 30-second sit-to-stand test; 6MWT, 6-minute walk test; 6MMT,6-minute mastication test; ASM, Appendicular muscle mass; FMI, fat mass index; FFMI, fat free mass index; LTMI, lean tissue mass index; SMI, skeletal muscle index. *P-values ≤0.02 (**in bold**) were considered significant. 1) too low handgrip strength = <16 kg for women and < 27 kg for men based on de Dodds reference at age 70 [29] according to the sarcopenia consensus 2018 [22]. 2) too low handgrip strength based on de Dodds reference [29] according to actual age.3) high fat percentage according to the sarcopenic obesity criteria of Baumgartner = >28% for men and >40% for women [40]. 4) too low SMI <7.0 kg/m^2 for men and <6.0 kg/m^2 for women according to the recommendations from European Working Group on Sarcopenia in Older People 2 (EWGSOP2) [22, 30].5) Ostopenia = t score between -1 and -2,5.6) too low protein intake = <1,2 gram/kg/day for MD patients at risk for malnutrition = PG-SGA≥ 4 and/or malnutrition according to GLIM criteria (n=34; 92% off MD patients) and 0.8 gam/kg/day for controls and MD patients not at risk for malnutrition. 7) to low Kcal intake <90% of calculated energy needs = resting energy expendure according to the Harris and Benedict formula (1984) and an activity factor of 1.4 for mobile MD patients, 1.2 for immobile MD patients and 1.5 for controls [10]. 8) PG-SGA, Patient-generated subjective global assessment: 0-1 does not require nutritional intervention, 2-3 requires nutritional education, 4-8 requires specialized nutritional intervention, ≥9 in critical need of symptom management together with specialized nutritional intervention/malnutrition [39].9) Malnutrition and severe malnutrition according to GLIM criteria [30].10) Sarcopenia according to 2018 consensus [22]. 11) Sarcopenic obesity according to Baumgartner [40] low SMI and high fat percentage.*

Association between physical functioning and protein intake

Protein intake was inversely correlated with fat mass index in MD patients (Spearman r = - 0.55, p < 0.01) and controls (Spearman r = -0.39, p = 0.02) (Figure 2c). Physical function was increased with higher protein intake in MD patients as measured by the 30SCT (r = 0.57, p = 0.006) (Figure 2d) and handgrip strength (Spearman r = 0.39, p = 0.02) (Supplement 2), but not in controls.

Other associations

Protein intake and handgrip strength correlated with arm lean tissue mass index in MD patients and controls, respectively (MD patients: Pearson r = 0.53, p <0.01 and controls: Pearson r = 0.81, p <0.001). No correlations were found between either the 6MWT, nor the 6MMT and body composition in MD patients and controls. The 30SCT correlated with fat mass index (r = 0.41, p = 0.01), but not with fat free mass index (r = 0.01, p = 0.96) in controls solely. No correlations were found between the 30SCT and 6MWT with leg lean tissue mass index. 6MMT correlated moderately with protein intake (Spearman r = 0.45, p = 0.02) in MD patients (Supplement 2).

Malnutrition and sarcopenia

According to the PG-SGA, 32 MD patients (86%) needed a nutritional intervention, whereas, this was only the case in two controls (6%) (Table 3). According to the GLIM criteria 46% of the MD patients were malnourished, whereas 43% according to the

PG-SGA (Table 3). If the PG-SGA date were combined with the GLIM data 27 MD patients (73%) were classified as malnourished. Sarcopenia was observed in 14 or 27% of the MD patients using either the consensus criterion or the actual age cutoff point for low handgrip strength, respectively.

Diagnostic accuracy of BIA versus the DXA

Both BIA-derived fat free mass formulas of Kyle and Dey show good correlation with DXA fat free mass in MD patients (Kyle $r^2 = 0.9$, Dey $r^2 = 0.8$) as well as in controls (Kyle $r^2 = 0.94$). BIA tends to overestimate fat free mass compared to DXA (mean difference = 1.8 kg, $p = 0.01$; 95%CI: -3.8-7.4 kg). BIA sensitivity (66%) and specificity (57%) to diagnose obesity are lower than to diagnose malnutrition (sensitivity 77%, specificity 93%) (supplement 3).

DISCUSSION

The main finding of this study is that muscle strength is related with body composition and protein intake in MD patients.

Hou et. al. (2019) [12] found a positive correlation between skeletal muscle mass index and muscle strength (r = 0.4) which is consistent with our hypothesis but was not significant in our cohort, this may be due to smaller numbers. However, a positive association between skeletal mass index and handgrip strength was found in the m.3243A>G genotype subgroup (supplement 1) and a moderate association between handgrip strength and arm lean tissue mass index was found.

Surprisingly sarcopenia existed in MD patients only, while no difference in muscle mass between MD patients and controls was observed. This might imply that handgrip strength and body composition are differently associated in MD patients compared with controls. Our results confirm a different association in fat free mass index with handgrip strength between controls (Pearson r = 0.67, p < 0.001), and MD patients (Pearson r = 0.30, p = 0.08, Figure 2a).

A high prevalence of malnutrition in MD patients (73%) was confirmed [9]. According to the GLIM criteria 46% of the MD patients were malnourished, whereas 43% according to the PG-SGA (Table 3). This seems a consistent result, however, Figure 3 shows low comparability in malnutrition between the two methods. A better comparability was seen between sarcopenia and malnutrition since all patients diagnosed with sarcopenia were also classified as malnourished (Figure 3). This low comparability underlines the challenges of diagnosing malnutrition and the conclusions of Aubry et al. (2017) [9] that nutritional assessment should be part of patient care in all adult MD patients. For measuring body composition DXA should be preferably used instead of BIA because

of the higher accuracy. For physical functioning it is advisable to measure handgrip strength and use the actual age cutoff point of the Dodds reference [29]. The incidence of sarcopenia with this actual age cutoff point (27%) is very similar to the results of Hou et al. (2019) [12] who observed sarcopenia in 24.7% of the MD patients. The risk for malnutrition according to the PG-SGA is higher in the current study compared to the slightly overlapping cohort from the DINAMITE study [27] 89% versus 55%. The DINAMITE study-cohort consisted of outpatients whereas the DYNAMO study-cohort was more severely affected that justified a four-day hospital admission. According to Hou et. al. [12] body composition is a sensitive biomarker of disease severity in MD patients. As low muscle mass is an indicator for malnutrition the difference in malnutrition risk observed in the two studies is probably due to differences in disease severity.

The observed decreased physical functioning in MD patients confirms previous studies [12].

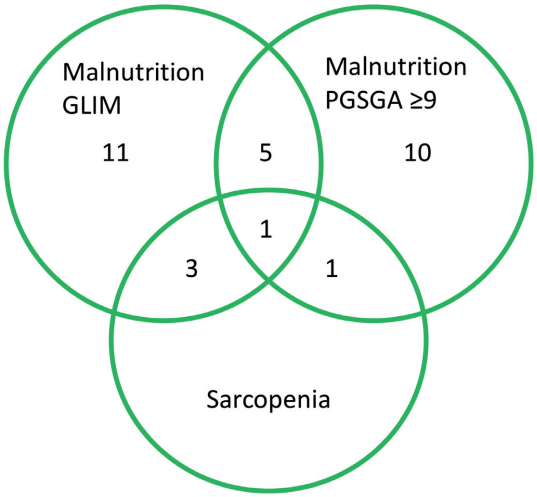

Figure 3. Venn diagram malnutrition according to GLIM criteria and PG-SGA score ≥ 9 and Sarcopenia consensus 2018.

Except for lower statue, lower appendicular skeletal muscle mass and lower leg lean tissue mass index, body composition in MD patients was not different compared to controls. Although not significant and less profound, our data showed a trend towards higher fat mass and lower lean mass in MD patients, which is similar to other studies [9, 12]. In the study of Aubry et al. (2017) [9], MD patients had higher BMI with comparable weight than MD patients in the current study. Moreover, that MD-patient group consisted of more males and had a higher mean age compared to the current study [9]. As height and body composition are known to be gender- and age-related [7, 13] this may have contributed to the (larger) difference in body composition observed in the study of Aubry et al. (2017) [9]. When comparing the individual MD patients and controls' body composition in the current study with reference values [41], a low skeletal muscle

index in both MD patients and controls (68% versus 57%) was observed. This result was anticipated for the MD patients, but not for the controls, and might indicate that either the controls do not represent a healthy status of body composition or the reference data are not representative for the healthy Dutch population.

A lower protein intake (g/kg/d) could not be confirmed in MD patients compared to controls ($p = 0.07$). However, relative to their protein needs, more MD patients have a protein intake that is too low (68%) compared to controls (12%).

The matched-control design, together with the accurate measurement of whole and regional body composition using DXA are strengths of this study. The small study sample size is a shortcoming. However, taking into account the low prevalence of MD, a total of 37 MD patients is considerable.

Data collection bias may have occurred due to having multiple professionals performing the measurements, although standard operating procedures have been applied. Validity of the DXA measurements may be influenced by the use of two scanners. However, that the body composition was not different in MD patients compared to controls could not be explained by this, since the body composition measured by BIA confirm that there was no difference between body composition in MD patients compared to controls (supplement 3). Furthermore, underreporting or overreporting of nutritional intake might have occurred.

Causal relationships could not be established in this study. The associations observed were moderate at best, indicating confounding or influencing factors. Handgrip strength is known to be dependent by physical strength exercise and also gender and age play a big role [42]. Gender and age cannot be changed but exercise and nutrition intake are something that can be influenced.

Although fat mass did not differ between MD patients and controls, MD patients frequently have a high fat percentage (24%, Table 3) and high waist circumference (38%, Table 1). As patients with neuromuscular disease are at risk for developing metabolic syndrome [43], monitoring body composition is recommended. If DXA is not available and as BIA has low sensitivity and specificity of diagnosing high fat percentage (supplement 3) waist circumference in MD patients is the preferred method. Nutritional strategies aiming at improving physical functionality and preventing metabolic syndrome are recommended. Improving protein intake seems a good start because a positive moderate association between protein and muscle strength was found as well as a moderate negative association between fat mass and protein intake. Prospective intervention studies to investigate if adjusting protein intake may improve functioning and muscle mass are recommended.

CONCLUSION

In conclusion, MD patients have decreased physical functioning. There is a moderate inverse association between handgrip strength and fat mass index in MD patients and controls, between handgrip strength and fat free mass index only in controls and between protein intake and muscle strengths in MD patients only. This, in combination with the high prevalence of both malnutrition and sarcopenia, warrants nutritional assessment in MD patients. Future intervention studies on improving protein intake are recommended.

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CONFLICTS OF INTEREST STATEMENT

No conflict of interest

FINANCIAL DISCLOSURE

None declared

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SUPPLEMENT 1

Correlations between HGS and body composition (FFMI, SMI and FMI) in different genotypes of mitochondrial diseases.

Genotype		FMI	FFMI	SMI
m.3243 A>G	*r*	**-0.60**	0.38	**0.44**
	p-value	**0.001**	0.04	**0.018**
	n	29	29	29
Others (n=5 point mutations (mtDNA), n=2 nDNA mutation and n=1 deletion mtDNA)	*r*	-0.60	-0.12	-0.13
	p-value	0.117	0.778	0.756
	n	8	8	8
Total group MD patients	*r*	**-0.61**	0.28	0.31
	p-value	**< 0.001**	0.096	0.064
	n	37	37	37

*FFMI, fat free mass index (kg/m^2); FMI, fat mass index (kg/m^2); HGS, handgrip strength (kg); r, Spearman correlation P-values < 0.02 (***in bold***) were considered significant.*

We tested the correlation between handgrip strength and body composition for genotype and categorized it into groups with a minimal of 8 patients and used spearman correlations for all tests because of small subgroups.

SUPPLEMENT 2

Correlations between body composition, physical functioning and protein intake.

		FFMI		**FMI**		**Arm LTMI**		**Leg LTMI**		**Protein intake**	
		Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control
HGS	*r*	0.30	**0.67**	**-0.61**	**-0.49**	**0.53**	**0.81**			**0.39**	-0.14
	p-value	0.08	**<0.01**	**<0.001**	**0.002**	**<0.01**	**<0.001**			**0.02**	0.42
	n	37	**37**	**37**	**37**	**37**	**37**			**37**	37
6MWT	*r*	-0.26	-0.25	-0.19	-0.25			-0.1	0.22	0.35	-0.26
	p-value	0.12	0.13	0.43	0.13			0.97	0.19	0.13	0.12
	n	20	37	20	37			20	37	20	37
30SCT	*r*	-0.42	0.01	-0.29	**0.41**			-0.27	-0.16	**0.57**	-0.12
	p-value	0.05	0.96	0.20	**0.01**			0.23	0.35	**0.006**	0.50
	n	22	36	22	**36**			22	36	**22**	36
6MMT	*r*	0.25	0.20	-0.07	0.22					**0.45**	-0.21
	p-value	0.19	0.25	0.69	0.20					**0.02**	0.23
	n	29	36	29	36					**29**	36
Protein intake	*r*	-0.19	-0.13	**-0.55**	-0.39	-0.04	0.06		-0.03		
	p-value	0.27	0.94	**<0.0001**	0.02	0.84	0.71		0.85		
	n	37	37	**37**	37	37	37		37		

*FFMI, fat free mass index (kg/m2); FMI, fat mass index (kg/m2); LTMI, lean tissue mass index (kg/m2); HGS, handgrip strength (kg); 6MWT, 6-minute walk test (total distance walked in meters); 30SCT, 30-second sit-to-stand test (total sit-to-stands movements); 6MMT, 6-minute mastication test (total chewing cycles); protein intake in g/kg/d; r, Spearman correlation for FMI, Protein intake and 6MWT, Pearson correlation for the rest. P-values ≤0.02 (***in bold***) were considered significant.*

SUPPLEMENT 3

Diagnostic accuracy of BIA versus DXA in adult mitochondrial disease patients
36 MD patients (age 42 ± 12 years, 40% males) +37 matched controls

Table 1. Difference FFMI and FMI between BIA and DXA.

	FFMI M±SD (kg/m²)			FMI Median IQR (kg/m²)		
	MD patients	**controls**	**P value**	**MD patients**	**controls**	**P value**
DXA	15.2± 1.9	15.5 ± 1.6	0.38	7.7 :6.7-8.7	7.0 :6.3-7.7	0.25
BIA	15.9 ± 1.7	16.7 ±1.6	0.04	6.9:5.9-8.0	6.3:5.8-7.2	0.53
P value	**<0.001**	**<0.001**		**<0.001**	**<0.001**	

*BIA, Bioelectrical impedance analysis DXA, Dual-energy X-ray absorptiometry, M, mean; SD, standard deviation; IQR, interquartile range, FFMI, fat free mass index (kg/m²); FMI, fat mass index (kg/m²);P-values <0.02 (***in bold***) were considered significant.*

Table 2. Correlation FFM BIA formula Kyle and Dey versus DXA.

FFM	R^2	SEE (kg)	P value	Data source
Kyle healthy from literature [1] n=343	0.97*	1.8	**<0.0001**	Kyle et al (2001) [2] BIA = Xitron
Kyle controls n=37	0.94	1.9	**<0.0001**	Dynamo study
Kyle MD patients n=36	0.90*	2.6	**<0.0001**	Dynamo study[a]
Dey MD patients n=36	0.89*	2.4	**<0.0001**	Dynamo study

*BIA, Bioelectrical impedance analysis DXA, Dual-energy X-ray absorptiometry, FFM, fat free mass (kg); P-values <0.02 (***in bold***) were considered significant. SEE= standard error of estimate a) See Figure 1 Blant altman plot*

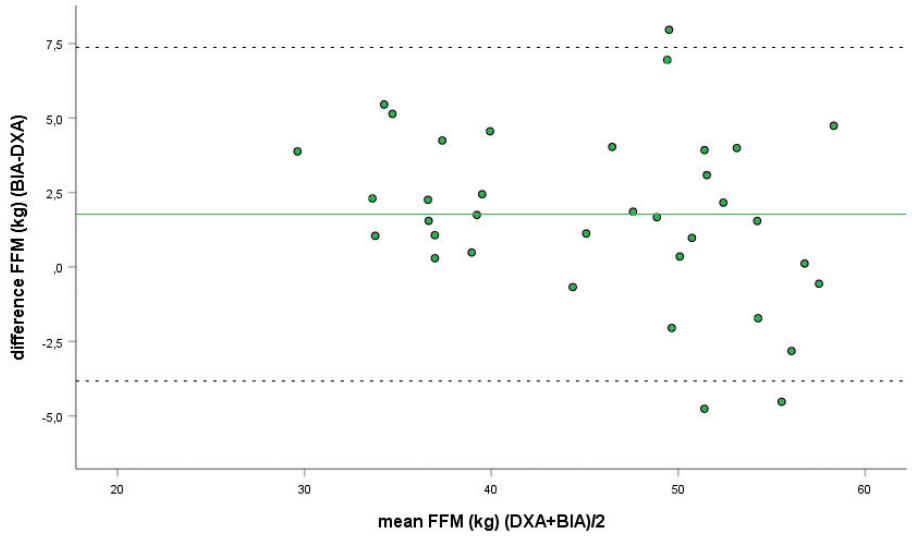

Figure 1. Blant altman plot FFM DXA versus BIA by Kyle in MD patients. BIA, Bioelectrical impedance analysis DXA, Dual-energy X-ray absorptiometry, FFM, fat free mass (kg)

Table 3. Sensitivity and specificity for diagnosing malnutrition/low FFMI by BIA versus DXA.

Malnutrition		**DXA**		Total
		Yes	No	
BIA	Yes	17 **(77%)** *	1 (7%)	18
	No	5 (23%)	13 **(93%)** **	18
Total		22	14	

= sensitivity=specificity; BIA, Bioelectrical impedance analysis DXA, Dual-energy X-ray absorptiometry, FFMI, fat free mass index (kg/m^2); Malnutrition is defined as FFMI< 15 kg/m^2 for women and < 17 kg/m^2 for men [3].*

Table 4. Sensitivity and specificity for diagnosing obesity/high fat percentage by BIA versus DXA.

Obesity		**DXA**		Total
		Yes	No	
BIA	Yes	19 **(66%)** *	3 (7%)	22
	No	10 (23%)	4 (**57%)** **	14
Total		29	7	

= sensitivity=specificity; BIA, Bioelectrical impedance analysis DXA, Dual-energy X-ray absorptiometry, obesity defined as fat percentage > 30% for women and >25% for men [4].*

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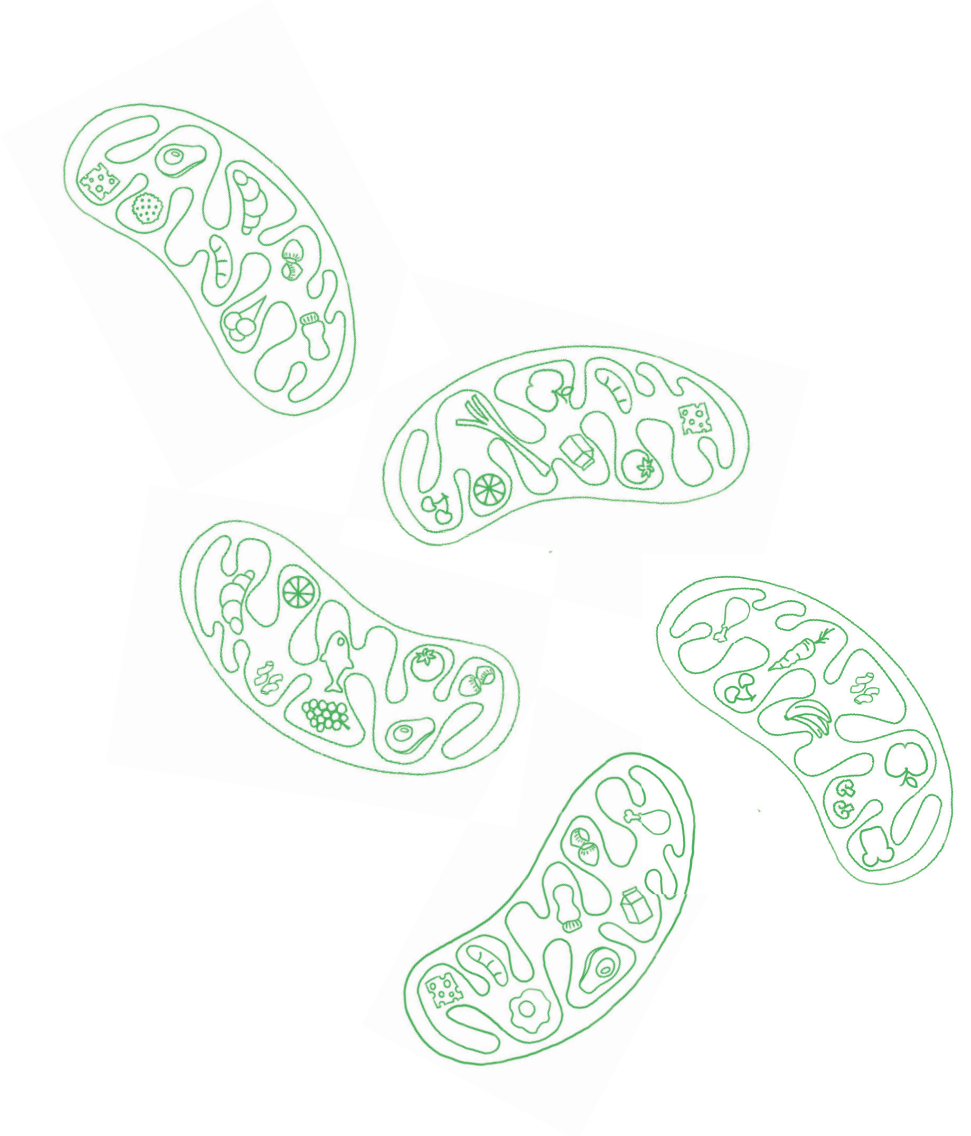

CHAPTER 5

The optimal estimate for energy requirements in adult patients with mitochondrial disease

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ABSTRACT

Aim

We aimed to identify the optimal method to estimate total energy expenditure (TEE) in mitochondrial disease (MD) patients.

Methods

Resting energy expenditure (REE) was measured in MD patients carrying the m3243A>G mutation using indirect calorimetry (IC) and compared with results of 21 predictive equations (PEs) for REE and with REE-IC measurements in healthy controls. Physical activity level (PAL) was measured using accelerometry (Sensewear®) and compared with a fixed average PAL (1.4) as well as patients' self-estimated activity levels. TEE was calculated as REE-IC × PAL Sensewear® and compared with usual care and energy recommendations for healthy adults.

Results

Thirty-eight MD patients (age: 48 ± 13 years; body mass index 24 ±4 kg/m^2; male 20%) and 25 matched controls were included. The accuracy of most PEs was between 63% and 76%. The difference in REE-IC in healthy controls (1532±182 Kcal) and MD patients (1430±221 Kcal) was borderline not significant (P=0.052). Patients' estimations for PAL were 18%-34% accurate at the individual level. The fixed activity factor was 53% accurate. Patients overestimated their PAL. Usual care predicted TEE accurately in only 32% of patients.

Conclusion

TEE is lower in these MD patients than the recommendations for healthy adults because of their lower physical activity. In MD patients, 6 PEs for REE provide a reliable alternative for IC, with an accuracy of 71%-76%. As PAL is highly variable and not reliably estimated by patients, measurement of PAL using accelerometry is recommended in this population.

Clinical Relevancy Statement

This study provides innovative information on total energy expenditure which can help guide individual dietary treatment of patients with mitochondrial disease. Energy requirements are very relevant for this patient population since the energy production in these patients is decreased.

INTRODUCTION

Mitochondria are responsible for the transformation of energy from nutrients into adenosine triphosphate (ATP), through oxidative phosphorylation (OXPHOS). Mitochondrial dysfunction can result from mutations in either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA). The incidence of primary mitochondrial disorders is approximately 1:5000 of all live births [1]. The most frequently reported pathogenic mitochondrial mutation in adults is the m.3243A>G point mutation [1, 2]. The most frequently reported phenotype of the m.3243A>G mutation is Maternal Inherited Diabetes and Deafness (MIDD) but also Mitochondrial Myopathy is very common. Mitochondrial Encephalomyopathy and Lactate Acidosis and Stoke-like episodes (MELAS) is a more severe phenotype with a lower incidence. Frequently occurring symptoms are hearing loss, gastro-intestinal symptoms, exercise intolerance, diabetes and myopathy [2]. Since specific treatment options remain limited, treatment is based on symptoms' management like exercise [3] and nutritional care [4].

Accurate prediction of total energy expenditure (TEE) is crucial to guide individual dietary treatment [4] since MD patients are both at risk for malnutrition [5-7] and overnutrition with a higher risk for comorbidities and metabolic syndrome [4, 8, 9].

At this point, it is not clear if and how the mitochondrial disease affects energy requirements. Defects in the oxidative phosphorylation system (OXPHOS) could theoretically lead to a lowered oxygen consumption rate at the cellular level [10] with lower REE. A lower fat free mass such as is frequently seen in MD [4-7] might also lower the REE [11]. Previously, Fiuza-Luces et al (2016) did not find a lower VO2 or lower REE when assessing Indirect Calorimetry in 20 children with MD [12]. On the other hand, the lactic acidosis that frequently occurs in mitochondrial disease may induce increases in the volume of CO2 production based on exhaled CO2 (VCO2). This could mean a higher REE. The latter could not be confirmed by Fiuza-Lucas et al (2016), although these authors reported a nearly significant (p=0.086) increase in REE in children with MD as compared to healthy controls.

Mitochondrial Diseases are a heterogeneous patient group in whom nutritional intake tends to be inadequate [13]. Measuring REE using indirect calorimetry is recommended [5, 12], but this is a relatively expensive method that requires the presence of trained personnel. For this reason, REE is often estimated in an indirect manner by means of predictive equations [14]. It is known that these predictive equations are not very accurate at individual level [14] but how accurate they are in mitochondrial disease is not known.

Physical activity has a major influence on total energy expenditure [15] and it is known that patients with MD are less active than healthy controls in this respect [8, 16]. Physical

activity can be reliably measured using non-invasive accelerometery [17]. This, however, is not implemented in most clinical settings and in usual care PAL is mostly estimated based on anamnestic data obtained from patients or by the use of standard PAL based on literature [8] or protocols [11, 18].

The aim of this study was to identify the optimal method to estimate total energy expenditure in MD patients. Also, we wanted to learn whether the energy requirements in adult MD patients differ from healthy adults.

MATERIALS AND METHODS

Standard protocol approvals, registrations and patient consents

This study was conducted in accordance with good clinical practice and the Declaration of Helsinki. The Medical Ethics Committee of the Arnhem and Nijmegen region approved the study protocol (NL 39724.091.13/2013/146) and written informed consent was obtained from every patient.

Study design/patients

In this study we used baseline data of the DINAMITE study, a randomized controlled trial with an individual dietary intervention in adult patients with MD due to the m.3243A>G mutation [4], as well as healthy control data from the DYNAMO study, a cross-sectional study on the association of body composition, physical functioning and protein intake in adult patients with mitochondrial diseases [7].

Thirty-nine patients with MD due to the m.3243A>G mutation from the Radboudumc patient cohort were included in the DINAMITE study. These could participate when they were at least 18 years of age, had no medical contraindication to undergo Nutritional Assessment after overnight fast, had no cardiac pacemaker nor did they suffer from claustrophobia. Patient measurements were performed between 2014 and 2017. Maternal lactation was another exclusion criterium for this study. Seventy-three healthy controls were included in the DYNAMO study: we used only the data of controls that on average matched for gender, age (± 2 years) and BMI (± 1 kg/m^2).

Phenotype, the Newcastle Mitochondrial Disease Adult Scale (NMDAS) [19] and heteroplasmy levels of the mutation measured in urinary epithelial cells (UEC) [20] were collected from patient files.

Anthropometrics

Height (cm) and weight (kg) were measured. The Fat Free Mass (FFM) was estimated by single frequency bioimpedance analysis (BIA) (Bodystat 1500 MDD) at 50 Hz, FFM (kg) was calculated according to the formula presented by Kyle [21].

Resting energy expenditure

As gold standard for Resting Energy Expenditure (REE) (Kcal) Indirect Calorimetry (IC) was measured as assessed for 20-30 minutes with the Cosmed Quark RMR® lying down with the canopy after an overnight fast according to the Dutch national Standard Operating Procedure. The REE-IC was compared to the results of 21 predictive equations, including 4 that incorporate FFM (See Supplement 1), and to the REE-IC of healthy controls.

Physical activity level

Physical activity was measured using a validated multi-sensor actometer [22-25] (Sensewear®, Bodymedia with Sensewear pro algorithm version 5.2), which was worn over a 7-day period. Patients worn the device day and night they only must take it off for showering.

Physical activity data are presented as average METS/day, where 1 MET = resting metabolic rate. Physical Activity Level (PAL) is defined as the total energy expenditure/ resting energy expenditure. Therefore, average METS were interpreted as PAL and used as gold standard.
The gold standard was compared to:

1. Patients estimated activity level scored by the Dutch activity Table (see Table 1)
2. Usual care using a fixed PAL of 1.4. This is chosen for 2 reasons:
    a. Apabhai et al (2011) found in 100 genetically proved adult MD patients with Sensewear® accelerometery an average PAL of 1.4 [8]: this result is consistent with the average PAL in the DINAMITE study [4].
    b. Literature on PAL in patients with various diseases advise to consider a PAL of 1.3-1.5. This value is therefore commonly used in dietary practice, including in our centre [18].
3. Average PAL in healthy Dutch adults is considered to be 1.7 in persons up to 50 years of age and 1.6 from 50 years and older [26]. The recommended PAL is higher: 1.9-1.8 [26].

Total energy expenditure

We compared our gold standard for total energy expenditure (= REE IC x PAL Sensewear®) to usual care and energy recommendations for healthy adults. The usual care at our centre is to use the WHO formula with weight and height [14] as prediction equation for REE for patients with a BMI <30 kg/m^2 and Harris-Benedict (1918) [27] for patients with BMI ≥ 30 kg/m^2 based on the study of Kruizinga et al (2016) [14]. This estimated REE is multiplied with the usual care fixed PAL of 1.4 to calculate the usual care total energy expenditure. The energy recommendations are based on the WHO REE prediction equation + weight multiplied with a PAL of 1.7 < 50 year and 1.6 from 50 years and older [26].

Table 1. Activity level scored according to criteria from the Dutch Health Council (Gezondheidsraad) [26] translated into lower and upper PAL values as described by Black (1996) [15], based on double-labelled water measurements.

Activity level according to Black (1996) [15]	**Answer Dutch activity table** [26]	**Lower PAL**	**Upper PAL**
Chair-bound or bed bound	I use a **wheelchair** all the time.	1.2	1.2
Seated work with no option of moving around and little or no strenuous leisure	I have **limited activity**, I alternate sitting with light housework and activities such as writing, washing	1.4	1.5
Seated work with discretion and requirement to move around but little or no strenuous leisure activity	I'm **moderately active**, I alternate sitting with light and heavier housework, gardening, walking, cycling, sports	1.6	1.7
Standing work (e.g. housewife, shop assistant)	I'm **normally active**, I don't sit very often	1.8	1.9
Strenuous work or highly active leisure	I'm **very active**, I do heavy physical work and/or I exercise a lot	2.0	2.4

PAL, physical activity level.

Statistics

Data were reported as means ± standard deviation (SD), or frequencies and percentage of the group or total population, if applicable. Normal distribution of the variables was assessed by Shapiro-Wilk tests. Differences between MD patients and controls were tested using the independent t-test. Differences between estimated PAL and measured PAL and between gold standard TEE with energy recommendations were tested using the paired t-test. Accuracy of predictions for REE /PAL/TEE were evaluated as percentage of subjects predicted within ±10% of measured REE/PAL/TEE, root mean squared error (bias) and mean absolute percentage difference between predicted and measured REE/PAL/TEE. Statistical analyses were performed using SPSS statistics (IBM Statistics 23). Two-sided testing was used in all cases and the significance level was set at $P < 0.05$.

RESULTS

Thirty-eight MD patients (age: 48±13 years; BMI 24 ±4 kg/m^2; male 20%; patients characteristics shown in Table 2) and 25 matched controls (age: 46 years ±11; BMI 24 ±3 kg/m^2; male 20%) were included. 1 patient from the DINAMITE study was excluded because she was lactating. Twelve controls from the DYNAMO were excluded because they did not match for gender, age and/or BMI.

Resting energy expenditure

Table 3 shows statistics of the 21 REE predictive equations for the MD patients. The accuracy of most (n=15) predictive equations was between 63-76%. Three out of four predictive equations that use fat free mass (FFM) as a variable were below 50% accurate. The three best scoring REE formulas were Henry based on weight and height [28], Muller using FFM [29] and Harris & Benedict (1984) [27]. The difference in REE-IC in healthy controls (1532 ±182 Kcal) and MD patients (1430 ± 221) almost reached statistical significance (p=0.052) see Figure 1.

Physical activity level

The patient's estimation for PAL were 18%-34% accurate at the individual level. The fixed activity factor (1.4) was 53% accurate (see Table 4). Patients overestimated their physical activity level. The difference between measured and estimated PAL was significant (p = 0.001 for the lower estimated PAL and p= <0.001 for the higher estimated PAL).

Total energy expenditure

Usual care accurately predicted TEE in only 32% of patients (see Table 5). Total energy requirements of MD patients were significantly lower than Dutch energy recommendations (p<0.0001).

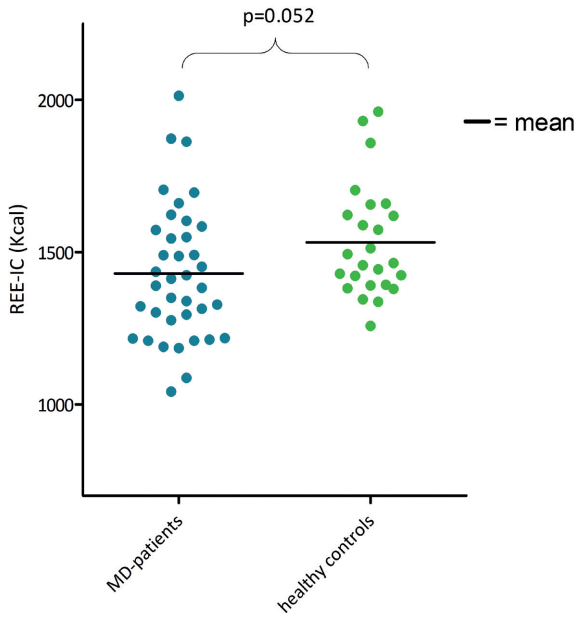

Figure 1. Resting Energy Expenditure measured by Indirect Calorimetry in Mitochondrial Disease patients and healthy controls. MD-Patients = Mitochondrial Disease Patients REE-IC = Resting Energy Expenditure measured by Indirect Calorimetry.

Table 2. Patients characteristics.

Variable	MD patients (n=38)
Age (years)	48 ±13
Female Gender	31 (82)
BMI (kg/m²)	24 (±4.2)
Underweight (BMI ≤ 18.5kg/m²)	2 (5)
Normal (BMI 18.5-25 kg/m²)	21 (55)
Overweight (BMI 25-30 kg/m²)	12 (32)
Obesity (BMI ≥30 kg/m²)	3 (8)
NMDAS score	18 ± 10
Heteroplasmy UEC	48 ± 22
Phenotypes	
MIDD	20 (53)
Myopathy	15 (42)
MELAS	2 (5)
METS Sensewear® = PAL	1.4 ± 0.25
FFMI (kg/m²) *	15.7 ± 2.3
Low n (%)	18 (47)
Normal n (%)	20 (53)
Self-estimated activity	
Limited active	11 (30)
Moderate active	17 (46)
Normally active	7 (19)
Very active	2 (5)
Wheelchair use	
I don't use a wheelchair	34 (92)
I only use a wheelchair outside with long distances	3 (8)
Activity stimulating factors	
No activity stimulating factors	16 (43)
Rehabilitation program	3 (8)
Physiotherapy	8 (22)
Sports	12 (32)

BMI, body mass index; FFMI, fat-free mass index; MELAS, mitochondrial myopathy, encephalopathy, lactate acidosis, and stroke-like episodes; METS, metabolic equivalents; MIDD, maternally inherited diabetes and deafness; NMDAS, Newcastle Mitochondrial Disease Adult Scale; UEC, urinary epithelial cells; Data shown as mean ± SD, number (%) low or normal FFMI as compared to reference values by Pichard et al (2004) [31]*

Table 3. Comparison 21 REE predictive equations with gold standard.

REE prediction equations	REE (kcal/ day)	SD (±)	Mean Absolute percent error (%)	Bias (kcal/ day)	% of MD patients with accurate estimation[a]	% of MD patients with under estimation[b]	% of MD patients with over estimation[c]
Gold standard = REE IC	1430	221	-	-	-	-	-
Henry H+W [25]	1378	189	**7.6****	164	**76***	21	3
Muller A [26]	1376	184	**7.8*****	163	**74****	24	3
H&B1984 [23]	1393	178	**7.8*****	**159****	**71*****	18	11
Henry H [25]	1356	176	7.9	174	**71*****	26	3
Cole [34]	1345	154	**7.6****	174	**71*****	29	0
Muller FFM [26]	1369	166	**7.5***	**160*****	**71*****	24	5
H&B1918 [27]	1398	171	7.9	**158***	68	21	11
Muller B [26]	1367	210	8.9	182	68	26	5
WHO W [11]	1422	176	8.4	162	66	16	19
Mifflin1 [30]	1324	199	8.7	192	66	34	0
Mifflin2 [30]	1320	200	8.6	195	66	34	0
Schofield H+W [31]	1401	175	8	167	66	21	13
Schofield W [31]	1418	172	8.2	162	66	16	18
WHO H+W [11]	1423	175	8.4	**158***	63	16	21
Owen [28, 29]	1317	172	9.1	200	63	37	0
Luis [32]	1474	200	10.6	197	53	13	34
Mifflin FFM [30]	1255	168	11.7	214	45	55	0
Weijs [14]	1560	204	13.5	215	37	5	58
Owen FFM [28, 29]	1194	194	16.2	273	21	79	0
Bernstein [33]	1148	130	18.9	325	16	84	0
Bernstein FFM [33]	1068	171	25.1	383	0	100	0

*REE= resting energy expenditure, PE= Predictive equation, IC= Indirect Calorimetry, SD=standard deviation, Bias= root mean squared error, H= Height, W= Weight, FFM= Fat Free Mass, a) Accurate estimation= REE PE between 90-110% of REE IC, b) Under estimation= REE PE <90% of REE IC· c) Overestimation= REE PE >110% of REE IC, * Best outcome, ** Second best outcome, *** Third best outcome*

Table 4. Comparison estimated PAL with gold standard.

	PAL	SD [b] (±)	Mean Absolute percent error (%)	Bias	% of MD patients with accurate estimation[a]	% of MD patients with under estimation[b]	% of MD patients with over estimation[c]
Gold standard = PAL (Sensewear®)	1.4	0.24	-	-	-	-	-
Patients Estimated lower PAL	1.6	0.17	18	0.29	34	13	53
Patients Estimated upper PAL	1.7	0.21	24	0.38	18	11	71
Usual Care = Fixed average PAL	1.4	-	**13***	**0.24***	**53***	**29**	18

PAL= Physical Activity Level, SD= standard deviation, Bias= root mean squared error SW= Sensewear®
*a) Accurate estimation= estimated PAL90-110% of PAL (SW), b) Under estimation= estimated PAL< 90 of PAL (SW), c) Overestimation= estimated PAL> 110 of PAL (SW) * **Best outcome***

Table 5. Comparison of the gold standard of total energy expenditure in MD patients with usual care and the Dutch energy recommendations.

	TEE (kcal/day)	SD (±)	Mean Absolute percent error (%)	Bias (kcal/ day)	% of MD patients with accurate estimation[a]	% of MD patients with under estimation[b]	% of MD patients with over estimation[c]
Gold standard = REE (IC) x PAL (Sensewear®)	2058	414	-	-	-	-	-
Usual care = WHO [11] (if BMI <30 kg/m^2) or HB (1918) [27] (if BMI ≥ 30 kg/m^2) x 1.4	1985	243	18	422	32	37	32
Energy recommendations WHO + W [11] x 1.7 (if age <50 year) or x1.6 (if age>50 year)	2348	281	23	498	32	11	58

TEE= Total Energy Expenditure REE = Resting Energy Expenditure, W= weight H= Height SD= standard deviation, Bias= root mean squared error, PAL= Physical Activity Level, a) Accurate estimation= Estimated TEE 90%–110% of gold standard TEE. b) Underestimation= Estimated TEE <90% of gold standard TEE, c) Overestimation= Estimated TEE > 110% of gold standard TEE.

DISCUSSION

The main finding of the present study is that the method in usual care of estimating total energy expenditure in adult MD patients seems adequate in only 32% of all patients. While six available prediction equations for REE showed an accuracy of 71-76% and seem a relatively reliable alternative to indirect calorimetry. The patient's own estimations for physical activity level proved not to be a suitable alternative to accelerometery.

The REE-IC in healthy controls seemed slightly (±100 Kcal) lower in MD patients as compared to healthy controls (1430 ±221 Kcal in MD patients versus 1532 ±182 in controls), but this difference was not statistically significant (p=0.052). Fiuza-Luces et al (2016) found an opposite but also non-significant difference (p=0.085) in REE-IC in MD children versus controls [12]. Based on these limited data it seems fair to assume that REE of these MD patients does not substantially differ from healthy individuals and does not have clinical implications.

The accuracy of 6 predictive equations was between 71%-76% (see Table 2). This is significantly better compared to the maximally 49% accurate predictions of Kruizenga et al [14] in a large hospital patient cohort (n=513). This suggests that for most MD patients the use of one of these six prediction equations provides a relatively reliable alternative to indirect calorimetry. Yet, dietitians should be aware that predictive equations remain estimations. The predictive equations can be used as an aid to the initial energy estimation, which then should be corrected according to changes in the patient's nutritional status.

Patients estimations of physical activity level did not prove reliable alternatives for accelerometery, given their accuracy of only 18%-34%, with patients overestimating their physical activity level. It should be mentioned here that the activity table that we used, and which has been validated for healthy individuals does not seem suitable for patients with exercise intolerance. For example, patients perform even light household tasks at a slower pace and with lower intensity. Because of our results, we recommend using accelerometery in all adult MD patients as part of their nutritional assessment. This technique is not expensive and doable for patients, whereas the alternatives seem inaccurate. In case this is not possible, the use of a fixed PAL of 1.4 provides a more reliable alternative (53%) compared to the patients' self-estimated PAL.

The total energy expenditure of MD patients is lower than that of recommendations for healthy individuals and since there was no difference in resting energy expenditure this difference is mainly explained by their lower physical activity level. This finding is in line with previous research in MD patients by Apabhai et al (2011) [8]. Since inter-individual differences in total energy expenditure may be substantial in this very heterogeneous patient group, Nutritional Assessment is recommended for accurate estimates at the individual level [4-6].

The Sensewear® accelerometer has been reported to overestimate energy expenditure in healthy adults, especially at high intensities [25]. Although patients with a mitochondrial disorder do not perform much intense activities [30] and the 5.2 version of the Sensewear® pro algorithm that was used shows better validity for measuring energy expenditure than the older 2.2. version [24], this presents a methodological limitation. Another shortcoming is the use of BIA to evaluate body composition. This is a double indirect method and BIA is known to overestimate fat free mass compared with dual-energy x-ray absorptiometry (DXA) in mitochondrial patients [7]. Finally, the use of the healthy energy recommendations as a control for total energy requirements instead of using a healthy control group that had the same accelerometery + IC measurements is a limitation. The energy recommendations for healthy adults [26] are not very recent (2006) and based on local Dutch recommendations stemming from the international WHO recommendations which are even older (2001) [11].

CONCLUSION

Total energy expenditure in MD patients is lower than suggested by recommendations for healthy adults because of the lower physical activity of the former. In MD patients six predictive equations for REE present a relatively reliable alternative for indirect calorimetry measurements. As PAL is highly variable and not reliably estimated by patients themselves, measurement of PAL using accelerometery is recommended in this population. If measuring activity is not feasible, the use of a fixed PAL of 1.4 is a more reliable method than using patient estimated activity levels.

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CONFLICTS OF INTEREST STATEMENT

No conflict of interest

FINANCIAL DISCLOSURE

None declared

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SUPPLEMENT 1

Predictive equations resting energy expenditure.

Name formula	Specificities	Formula
Harris & Benedict 1918 [1]	Men	66,4730 + (13,7516 x weight (kg)) + (5,0033 x height (cm)) - (6,7550 x age)
	Women	655,0955 + (9,5634 x weight (kg)) + (1,8496 x height (cm)) - (4,6756 x age)
Harris & Benedict 1984 [1]	Men	88,362 + (13,397 x weight (kg)) + (4,799 x height (cm)) - (5,677 x age)
	Women	447,593 + (9,247 x weight (kg)) + (3,098 x height (cm)) - (4,33 x age)
Weijs [2]	BMI <25kg/m^2	(11.355 x weight (kg)) + (7.224 x height (cm)) – (4.649 x age) + (135.265 x sexe) – 137.475
	BMI >25kg/m^2	(14.0238 x weight (kg)) + (4.498 x height (cm)) – (0.977 x age) + (137.566 x sexe) – 221.631
		Sexe male =1, female = 0
WHO-1985 [3]	Men 18-30 year	15,4 weight (kg) – 27 height (m) + 717
	Men 30-60 year	11,3 weight (kg) – 16 height (m) + 901
	Men >60 year	8,8 weight (kg) + 1128 height (m) - 1071
	Women 18-30 year	13,3 weight (kg) + 334 height (m)+ 35
	Women 30-60 year	8,7 weight (kg) - 25 height (m) + 865
	Women >60 year	9,2 weight (kg) + 637 height (m) - 302
WHO - 1985 weight [4]	Men 18-30 year	15,3 x weight (kg) + 679
	Men 31-60 year	11.6 x weight (kg) + 879
	Men >60 year	13.5 x weight (kg) + 487
	Women 18-30 year	14.7 x weight (kg) + 496
	Women 31-60 year	8.7 x weight (kg) + 829
	Women >60 year	10.5 x weight (kg) + 596
Owen [5]	Men	879 + (10,2 x weight (kg))
	Women	795 + (7.18 x weight (kg))
Owen FFM [5]	Men	22,3 × FFM + 290 = kcal/d
	Women	19,7 × FFM + 334 = kcal/d
Müller A [6]		0.047 x weight (kg) + 1.009 x sexe – 0.01452 x age + 3.21 = REE MJ/d
		sexe male = 1, female = 0

Predictive equations resting energy expenditure.

Name formula	Specificities	Formula
Müller B (BMI) [6]	BMI <18.5kg/m^2	0.07122 x weight (kg) – 0.02149 x age + 0,82 x sexe + 0.731 = REE MJ/d
	BMI >18.5kg/m^2	0.02219 x weight (kg) + 0.02118 x height (cm) + 0.884 x sexe – 0.01191 x age + 1.233 = REE MJ/d
	BMI >25 kg/m^2	0.04507 x weight (kg) + 1.006 x sexe – 0.01553 x age + 3.407 = REE MJ/d
	BMI >30kg/m^2	0.05 x weight (kg) + 1.103 x sexe – 0.01586 x age + 2.924 = REE MJ/d sexe male = 1, female = 0
Müller FFM [6]	BMI <18.5kg/m^2	0.08961 x FFM (kg) + 0.05662 x FM (kg) + 0.667
	BMI >18.5 tot 25kg/m^2	0.0455 x FFM (kg) + 0.0278 x FM (kg) + 0.879 x sexe - 0.01291 x age + 3.634
	BMI >25kg/m^2	0.03776 x FFM (kg) + 0.03013 x FM (kg) + 0.93 x sexe - 0.01196 x age + 3.928
	BMI >30kg/m2	0.05685 x FFM (kg) + 0.04022 x FM (kg) + 0.808 x sexe – 0.01402 x age + 2.818 Sexe male =1, female =0
Mifflin 1 [8]	Women	9.99 x weight (kg) + 6.25 x height (cm) – 4.92 x age -161
	Men	9.99 x weight (kg) + 6.25 x height (cm) – 4.92 x age + 5
Mifflin 2 [8]	Women	10 x weight (kg) + 6.25 x height (cm) – 5 x age – 161
	Men	10 x weight (kg) + 6.25 x height (cm) – 5 x age + 5
Mifflin FFM [8]	Men	22.5 x FFM (kg) + 209
	Women	20.8 x FFM (kg) + 369

Predictive equations resting energy expenditure.

Name formula	Specificities	Formula
Schofield height and weight [9]	Men 18-30 year	15.0 x weight (kg) – 10.0 x height (m) + 706
	Men 30-60 year	11.5 x weight (kg) - 2.6 x height (m) + 877
	Men >60 year	9.1 x weight (kg) + 972 x height (m) – 834
	Women 18-30 year	13.6 x weight (kg) + 283 x height (m) + 98
	Women 30-60 year	8.1 x weight (kg) + 1.4 x height (m) + 844
	Women >60 year	7.9 x weight (kg) + 458 x height (m) + 17.7
Schofield weight [9]	Men 18-29 year	2.84 + 0.0640 x weight (kg) = MJ/d
	Men 30-59 year	3.67 + 0.0485 x weight (kg) = MJ/d
	Men 60-74 year	2.93 + 0.0499 x weight (kg) = MJ/d
	Men >75 year	3.43 + 0.0350 x weight (kg) = MJ/d
	Women 18-29 year	2.08 + 0.0615 x weight (kg) = MJ/d
	Women 30-59 year	3.47 + 0.0364 x weight (kg) = MJ/d
	Women 60-74 year	2.88 + 0.0386 x weight (kg) = MJ/d
	Women >75 year	2.61 + 0.0410 x weight (kg) = MJ/d
Henry weight and height [10]	Men 18-30 year	14.4 x weight (kg) + 313 x height (m) + 113
	Men 30-60 year	11.4 x weight (kg) + 541 x height (m) + -137
	Men >60 year	11.4 x weight (kg) + 541 x height (m) + -256
	Women 18-30 year	10.4 x weight (kg) + 615 x height (m) + -282
	Women 30-60 year	8.18 x weight (kg) + 502 x height (m) + -11.6
	Women >60 year	8.52 x weight (kg) + 421 x height (m) + 10.7
Henry weight [10]	Men 18-30 year	16.0 x weight (kg) + 545
	Men 30-60 year	14.2 x weight (kg) + 593
	Men >60 year	13.5 x weight (kg) + 514
	Men 60-70 year	13.0 x weight (kg) + 567
	Men >70 year	13.7 x weight (kg) + 481
	Women 18-30 year	13.1 x weight (kg) + 558
	Women 30-60 year	9.7 x weight (kg) + 694
	Women >60 year	10.1 x weight (kg) + 569
	Women 60-70 year	10.2 x weight (kg) + 57
	Women >70 year	10 x weight (kg) + 577
Cole [11,12]	Men BMI 18-25 kg/m^2	$E^{-0.1631 - 0.00255 \times age + 0.4721 \times weight\ (kg) + 0.2952 \times height\ (m)}$ = REE MJ/day
	Men BMI 25-30 kg/m^2	$E^{-0.2630 - 0.00277 \times age + 0.4877 \times weight\ (kg) + 0.3367 \times height\ (m)}$ = REE MJ/day
	Women BMI 18-25 kg/m^2	$E^{-0.1934 - 0.00199 \times age + 0.4764 \times weight\ (kg) + 0.0194 \times height\ (m)}$ = REE MJ/day
	Women BMI 25-35 kg/m^2	$E^{-0.0713 - 0.00209 \times age + 0.4075 \times weight\ (kg) + 0.3540 \times height\ (m)}$ = REE MJ/day

Predictive equations resting energy expenditure.

Name formula	Specificities	Formula
De Luis [13]	Men	58.6 + (6.1 x weight (kg)) + (1,023.7 x height (m)) – (9.5 x age)
	Women	1,272.5 + (9,8 weight (kg)) – (61.6 x height (m)) – (8.2 x age)
Bernstein [14]	Men	11.02 x weight (kg) + 10.23 x height (cm) – 5.8 age (years) – 1032
	Women	7.48 x weight (kg) - 0.42x height (cm) -3.0xage (years) +844
Bernstein FFM [11]	Men/Women	19,02 × FFM + 3,72 × FM – 1,55 × age + 236,7 = kcal/d

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PART 2

Diet interventions

CHAPTER 6

Individual dietary intervention in adult patients with mitochondrial disease due to the m.3243A>G mutation: the DINAMITE study

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The Acronym DINAMITE stands for DIner Nutristional Assesment MITochondrial disorder Energy

ABSTRACT

Objective

To evaluate the effect of an individually tailored dietary intervention on personalized goals, body composition (BC), functioning, and quality of life (QoL) in adult patients with mitochondrial disease (MD) due to the m.3243A>G mutation.

Methods

In this explorative RCT 39 MD patients were included. The intervention group (n=20) received an individually tailored dietary intervention during six months. The control group (n=19) received usual care during six months (control period) followed by an individually tailored dietary intervention for the next six months (intervention period). Nutritional assessment and QoL measurements were performed at three-month intervals. Personalized treatment goals of the MD patients were evaluated at 3 and 6 months during dietary intervention. Achievement of the personalized goals was assessed using descriptive statistics and mixed models. Linear mixed models were used to test the effect of the dietary intervention on continuous outcomes.

Results

The personal goals of patients were significantly more frequent achieved in the intervention group compared to the control group. After 3 months of intervention 57% of the goals were achieved. Most goals were achieved for BC, HGS, and gastro-intestinal complaints. Intervention increased handgrip strength (HGS) (p=0.037), the vitality component of QoL (p=0.026), and decreased fatigue score (p=0.024) after three months of treatment. Effects did not seem to last after 3 months.

Conclusion

An individually tailored dietary intervention is promising to achieve personalized goals of patients with MD, especially with regard to BC, HGS, and gastro-intestinal complaints. The intervention also improves QoL and decreases fatigue.

INTRODUCTION

Mitochondrial diseases (MDs) are among the most prevalent inherited metabolic diseases, with an incidence of approximately 1:5000 live births [1]. The clinical presentation is heterogeneous. MD disorders can be classified according to whether the causative mutations affect mitochondrial DNA or nuclear DNA. The most frequently reported pathogenic mutation in adults is the m.3243A>G point mutation [1, 2]. Since specific treatment options remain limited, the management of mitochondrial disorders is mainly supportive.

Malnutrition frequently occurs in MD patients [3-5]. According to the definition by Stratton [6], the term 'disease related malnutrition' coins a shortage and/or disbalance of nutrient intake that leads to negative changes in BC, functioning, and clinical outcome. By this definition, the energy shortage in MD is malnutrition on a cellular level. It is suggested that malnutrition may worsen symptoms in MD, as for instance was shown in a study in young MD patients in which an association between nutritional status and mitochondrial functioning was observed [7]. Furthermore, it is known that malnutrition causes secondary mitochondrial dysfunction, and in this way, it may worsen complaints of MD patients [8].

Previous studies show that adult MD patients have an inadequate food intake with various shortcomings and excesses [3], in particular a low intake of protein and increased intake of saturated fat and sugar.

Changes in nutritional status in MD patients could be reflected in alterations in BC comparable with other neuromuscular disorders [9]. It has been shown that the body mass index (BMI) and fat free mass (FFM) in MD patients is lower than in the general population [4, 10].

With regard to functioning and clinical outcome, MD patients often complain about myopathy, fatigue, and exercise intolerance, along with an impaired QoL [11, 12]. It has been suggested that optimizing nutritional status may improve QoL of MD patients [5], as was shown in other settings such as cancer cachexia [13].

Until now, no effective treatment for MD exists. Moreover, proving positive effects of any kind of intervention on the clinical outcome in this disease is challenging, due to the small number of patients and the heterogeneity of the population [14]. Therefore, personalized intervention in these patients might be more appropriate. To date the influence of nutrition on the clinical outcome of MD patients has not been studied. Hence, this explorative randomized controlled trial (RCT) was conducted to explore the effect of an individually tailored dietary intervention on personal goals in adult mitochondrial patients carrying the m.3243A>G mutation. The effect of the dietary intervention on nutritional status; intake, BC, functioning and QoL was evaluated.

METHODS

Standard protocol approvals, registrations and patient consents

This study was conducted in accordance with good clinical practice and the Declaration of Helsinki. The Medical Ethics Committee of the Arnhem and Nijmegen region approved the study protocol and written informed consent was obtained from every patient. The study was registered as DINAMITE study in ClinicialTrials.gov (NCT02286856).

Study design

The DINAMITE study comprised a single center, explorative, 2-armed, randomized controlled dietary intervention study. Randomization for the intervention or control group was stratified for MD disease severity by the Newcastle Mitochondrial Disease Adult Scale [2]. An independent researcher prepared 2 sets of sealed envelopes one set for NMDAS ≥20 and one set for NMDAS <20; which were opened after patients signed informed consent.

The intervention group received an individually tailored dietary intervention for six months after randomization. The control group received usual care during the first six months (control period) immediately followed by individually tailored dietary intervention for six months.

Both the intervention and control group were evaluated at baseline (t=0), three (t=1), and six months (t=2). In addition, the control group was evaluated at nine (t=3) and twelve months (t=4) during their intervention period.

Patients

Eighty eligible patients with MD due to the 3243A>G mutation in mitochondrial DNA from the Radboud Centre for Mitochondrial Medicine cohort [2] were asked to participate in the study. They were allowed to participate when they were at least 18 years of age, had no medical contraindication to undergo Nutritional Assessment after overnight fast, had no pacemaker nor did they suffer from claustrophobia. The severely malnourished patients with a BMI below 17.5 were not included because of ethical considerations. Patients were measured between 2014 and 2017.

Dietary intervention

All patients received a written personal diet schedule in which the metabolic dietician made the translation from the nutrient requirements into food products. The diet schedule was based on a healthy diet with whole grain, low sugar and saturated fats. Personal food preferences were taken into account to enhance compliance. Energy advice was based on individually measured energy requirements. When patients wanted to lose weight the daily energy intake was reduced with ±250 Kcal a day to ensure slow weight loss en prevent loss of muscle mass. The energy restriction consisted out of cutting extras like sweets, alcohol and snacks. When patients wanted to gain weight a minimum of 250 Kcal a day was added combined with high protein and frequent meals, sometimes

with oral nutritional supplements. Individual protein requirements were calculated based on ideal body weight and nutritional status [15]. The patients with malnutrition based on low Fat Free Mass Index (FFMI) [16] were advised 1.2 grams of protein/kg ideal bodyweight. Patients with proteinuria were allowed a maximum of 0.8 grams of protein/kg bodyweight. Micronutrients were advised according to the RDA and if it was not possible to meet requirements with normal products supplements were advised. Constipation was treated with high liquid and high fibre diet. Patients with postprandial fullness, nausea and dysphagia were advised to adapt the consistency of nutrition to a more liquid form. This advice was also given to the patients who eat slow and had a low nutrient intake due to fatigue. In the patients with air related gastrointestinal problems (bloating, belching or flatulence) lactose and carbonic acid restriction was advised. In addition, practical advice was given on how to cook healthy when having less energy.

Personal goals

The personalized treatment goals were determined and evaluated by the patient in consensus with his/her dietician based on the results of the Nutritional Assessment. Goals of patients were determined for seven variables: weight, FFM, fat percentage, HGS, fatigue, gastrointestinal complaints, and other. Goal outcomes were defined as: "keep stable", "decrease/increase" or "not applicable". See supplement 1.

Measurements

All measurements were performed according to the corresponding standard operating procedures after an overnight fast. To evaluate protein intake, BC, and functioning, Nutritional Assessment was performed every three months. In addition, QoL was measured and the risk of malnutrition was assessed with the Patient-Generated Subjective Global Assessment (PG-SGA) [17]. NMDAS and heteroplasmy levels were collected from the patient files.

The following Intake and requirements were measured:

- Indirect Calorimetry (IC): at baseline, the resting energy expenditure (REE) [18] was measured by IC (Cosmed Quark RMR®).
- Physical activity Level (PAL): PAL was determined as the average total metabolic equivalents (METS) measured by an accelerometer (Sensewear mini ®) that was carried by the patients during one week [19].
- Nutritional intake: mean daily nutritional intake was calculated by means of a 3-day food record. The protein intake (g/day) was calculated in a standardized manner using the nutrition calculation program Madows® [3].

The following BC measurements were taken:

- Anthropometry: Height (cm), weight (kg), waist circumference (WC) (cm), triceps skinfold thickness (mm), upper arm circumferences (cm) and upper arm muscle circumference (cm) [20] were measured.

- BC: The FFM was estimated by single frequency bioimpedance analysis (Bodystat®) at 50 Hz, fat percentage, and fat free mass index (FFMI) were calculated with the formula of Kyle [21].

The following functioning parameters were measured:

- Objective variable hand grip strength: HGS (kg) was measured using the Jamar® dynamometer.
- Subjective variable fatigue: Fatigue was assessed with the short Checklist Individual Strength (CIS)-fatigue questionnaire [22].
- Gastrointestinal complaints: Nine questions of the gastrointestinal questionnaire were used [23]. Frequency of the symptoms was scored on a 7-point Likert scale.

The following QoL data were collected:

- The subjective QoL was assessed using the RAND-36 (SF-36) questionnaire [24]. This patient-reported survey provides a score for eight different dimensions: vitality, physical health, pain, general health, role functioning physical, role functioning emotional, social functioning, and mental health.

Data analysis

All data were collected on a paper case report form and doubly entered into the online Castor® database to minimize entry mistakes. Statistical analysis was performed using SPSS statistics (IBM, version 24).

Patient characteristics at baseline were compared with applicable references values [16, 17, 20, 25-28]. Baseline characteristics were reported as means ± standard deviation (SD) or as the exact number with percentage of the total population. Normal distribution of the variables was assessed by Shapiro-Wilk tests. Independent t-tests and Mann-Whitney U tests were performed at baseline to check for between group differences for continuous variables. Chi-Square test was used to test the distribution of categorical characteristics across the intervention and the control group.

The number of achieved personal goals was assessed using descriptive statistics. For each patient the number of goals that were defined at start and after 3 months of intervention was assessed, and how many goals were achieved after 3 and 6 months. For the whole population, the number of defined goals (in total and for each variable) and how many of these goals had been achieved was assessed. Because of the hierarchical structure of our study (personal goals nested within patient) we performed multilevel (mixed model) analyses. Difference in proportion of achieved goals between control and intervention was tested with a mixed model with a random intercept and all other variables fixed. The difference in proportion between control group and intervention group and difference in proportion within the control group between control period and intervention period were tested. For these tests it was assumed that the personal goals in the control period

were the same as the goals in the intervention period. In this analysis the gastro-intestinal and other goals could not be included because no data of achieving these goals in the control period was available.

Linear mixed models were performed using a time by group interaction to test the longitudinal effect of the dietary intervention on continuous outcomes: protein intake, weight, fat percentage, fat free mass index, upper arm muscle circumference, WC, HGS, fatigue, and the eight domains for QoL. Two different analyses were performed using mixed models analysis; first, the intervention group was compared with the control period of the control group (t_0-t_2; 6 months), and second the control period was compared to both the intervention group and the intervention period of the control group (t_0-t_4; 12 months). In the mixed models, the periods of the measurements and the control or intervention were fixed factors and subject was added as random factor. When graphs suggested a change over time, mixed model pairwise comparison of time points was performed as a secondary analysis.

Two-sided testing was used in all cases, the significance level was set at $P < 0.05$.

RESULTS

Eighty MD patients were approached, 40 were willing to participate in this study and signed informed consent (Figure 1). Patient characteristics are shown in Table 1 and were similar in the control and intervention group. For detail individual results see supplement 1. No harm or unintended effects were observed.

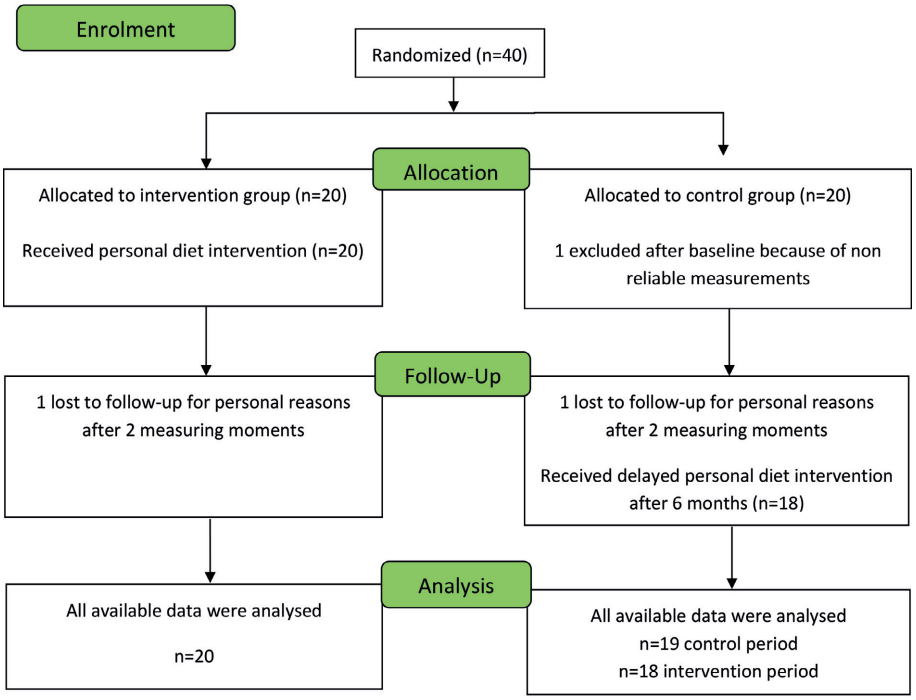

Figure 1. Flow diagram inclusion

Table 1. Patients characteristics.

Variable	Total population (n=39)	Intervention group (n=20)	Control group (n=19)
Age (years)	47 ± 13	47 ± 14	46 ± 12
Gender Female	32 (82)	16 (80)	16 (84)
BMI (kg/m^2)1	23.7 ± 4.7	25.0 ± 7.0	22.1 ± 5.6
Underweight	2 (5)	1 (5)	1 (5)
Normal	21 (54)	9 (45)	12 (63)
Overweight	12 (31)	7 (35)	5 (26)
Obesity	4 (10)	3 (15)	1 (5)
NMDAS score	18 ± 10	16 ± 10	19 ± 10
NMDAS ≥20	12 (32)	5 (26)	7 (37)
NMDAS <20	26 (68)	14 (74)	12 (63)
Heteroplasmy UEC	50 ± 21	45 ± 23	54 ± 19
Phenotypes			
MIDD	20 (51)	10 (50)	10 (53)
Myopathy	17 (44)	9 (45)	8 (42)
MELAS	2 (5)	1 (5)	1 (5)
Indirect Calorimetry (kcal)	1443 ± 240	1507 ± 266	1376 ± 194
Exercise METS	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.3
Sleep duration (h:mm)	7:41 ± 1:00	7:53 ± 1:07	7:29 ± 0:52
Energy intake (kcal)	1783 ± 453	1806 ± 478	1759 ± 437
Protein intake (gram)	71 ± 17	70 ± 14	71 ± 19
Low	14 (36)	8 (40)	6 (32)
Fat percentage (%)	34.8 ± 7.1	35.3 ± 7.0	34.2 ± 7.3
High	34 (87)	18 (90)	16 (84)
FFMI (kg/m^2)	15.7 ± 2.3	16.2 ± 2.3	15.3 ± 2.1
Low	20 (51)	9 (45)	11 (58)
Handgrip strength (kg)	24 ± 10	24 ± 8	24 ± 11
Low	33 (85)	18 (95)	15 (75)
UAMC (cm)	22 ± 23	22 ± 3	22 ± 3
Low	13 (33)	6 (30)	7 (37)
WC (cm)	90 ± 14	91 ± 14	88 ± 13
High	28 (72)	16 (80)	12 (63)
Fatigue score	40 ± 8	41 ± 9	38 ± 6
Fatigue	35 (90)	16 (84)	19 (95)
PG-SGA score	4 ± 3	4 ± 3	4 ± 3
risk for malnutrition	21 (55)	11 (58)	10 (53)

Data are shown as mean ±SD, number (%) or otherwise stated. [1]: shown as median ± IQR. BMI: Body Mass Index: Underweight <18.5 kg/m^2, Normal 18.5-24.9 kg/m^2, Overweight 25.0-29.9 kg/m^2, Obesity >30.0 kg/m^2, NMDAS: Newcastle Mitochondrial Disease Adult Scale, UEC: Urinary Epithelial Cells, MIDD: Maternally Inherited Diabetes and Deafness, MELAS: Mitochondrial myopathy, Encephalopathy, Lactate Acidosis, and Stroke-like episodes, METS: Metabolic equivalents, Protein Intake (g/day):% of the personally calculated needs were < 95% = low, ≥ 95% =adequate FFMI: Fat Free Mass Index (kg/m^2) [16], UAMC: Upper Arm Muscle Circumference (cm) [29], WC: Waist Circumference (cm) [26], HGS: Hand Grip Strength (kg) [28], PG-SGA: Patient Generated Subjective Global Assessment [17] 0-3 low risk for malnutrition ≥ 4= risk for malnutrition intervention is needed .

Goals

After three and six months of individually tailored dietary intervention significantly more goals were achieved in the intervention group compared to the control group (respectively OR 2.6 95% CI 1.4-5.1 and OR 2.0 95% CI 1.0-4.1) (Table 2). Within the control group no significant difference could be demonstrated between the control and intervention period (Table 3). A total of 182 individual goals were determined at the start of the dietary intervention with an average of five goals per patient. The majority of the goals (72%) aimed at changing an outcome variable, while the remainder aimed at stabilizing the variable. After three months of intervention 175 goals were defined for the whole patient group, of which 60% aimed to decrease or increase the variable, and 39% to keep the corresponding variable stable. In Figure 2A, the goals for the specific variables are shown. Most goals were achieved for BC, HGS, and gastro-intestinal complaints. In total, more goals were achieved after three months (57%) compared to six months (43%) (Figure 2B). All but one patient (97%) achieved at least one personal goal.

Table 2. Percentage achieved goals after 3 and 6 months: Comparison between control group and intervention group.

	intervention group n (%)	**control group n (%)**	**odds ratio (95% CI) P-value**
Achieved goals after 3 months	47 (52)	21 (29)	2.6 (1.4-5.1) **p= 0.004**
Achieved goals after 6 months	46 (52)	25 (35)	2.0 (1.0-4.1) **p= 0.002**

Table 3. Percentage achieved goals after 3 and 6 months: Comparison within control group between control period with intervention period.

	intervention period n (%)	**control period n (%)**	**odds ratio (95% CI) P-value**
Achieved goals after 3 months	26 (37)	21 (29)	1.4 (0.7-2.8) p=0.35
Achieved goals after 6 months	35 (47)	25 (35)	1.6 (0.8-3.1) p=0.17

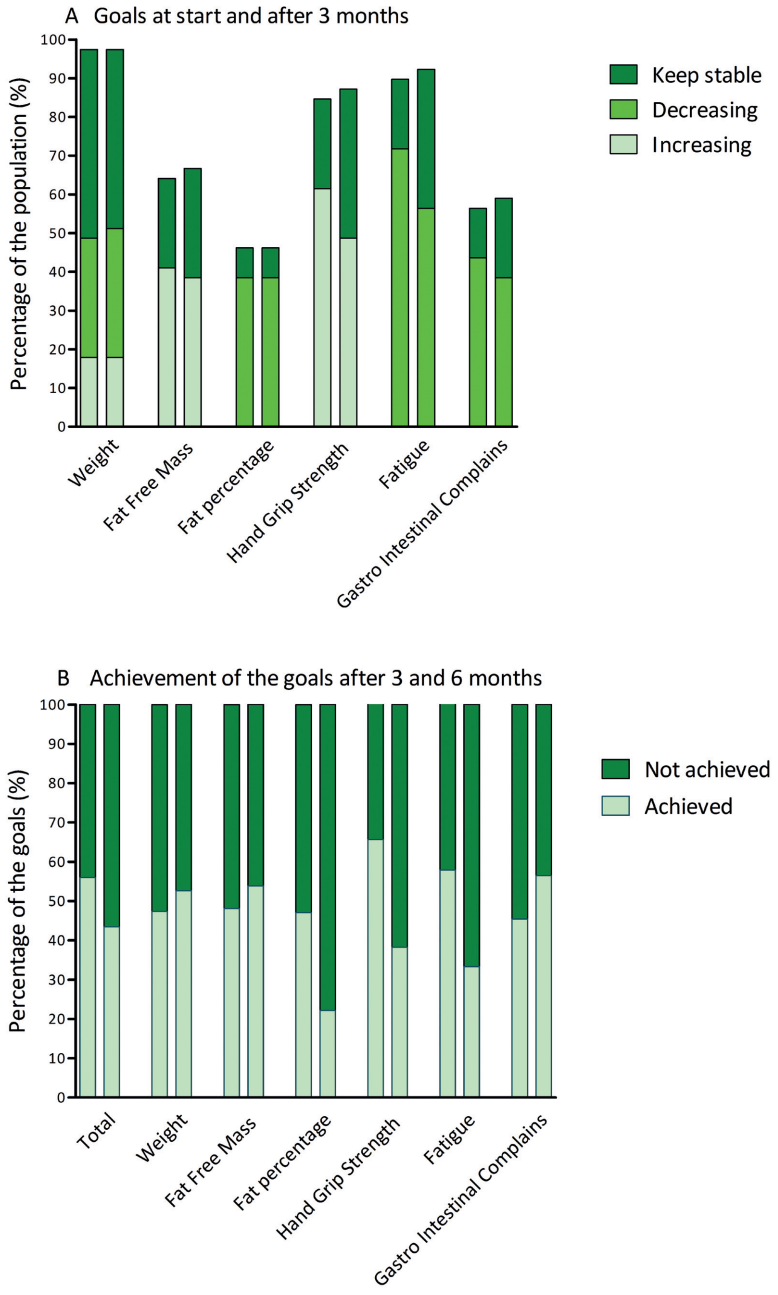

Figure 2. Analysis of the goals. Defined goals are presented as percentage of the population (A) at start (left) and after 3 months (right). Data about the achievement of the goals are presented as the mean percentage of the total goals of the corresponding variable (B) after 3 months (left) and after 6 months (right). n=38

Effect of individually tailored dietary intervention

Full data available in supplement 1.

Intake: No significant improvement was observed in protein intake (Figure 3A).

Body Composition: Personalized dietary intervention did not significantly affect body weight, fat percentage, FFMI, upper arm muscle circumference, or WC compared to the control group.

Functioning: The individually tailored dietary intervention increased HGS (p=0.037) in the intervention group compared to the control period (Figure 3B). Secondary pairwise comparison of time points showed that the HGS also increased (p=0.029) in the first three months of intervention for the control group but decreased again in the last three months of intervention (p=0.032). No significant change was observed for fatigue when comparing the intervention group with the control group (six and twelve months). The secondary analysis showed that fatigue score decreased (p=0.024) after three months of intervention in the intervention group compared with the control group (Figure 3C).

QoL: Vitality significantly improved between intervention group and control period (six months, p=0.026) and for the control period compared to intervention group and intervention period (twelve months, p=0.037) (Figure 3D). For the other domains for QoL no significant change was found (Supplement 2).

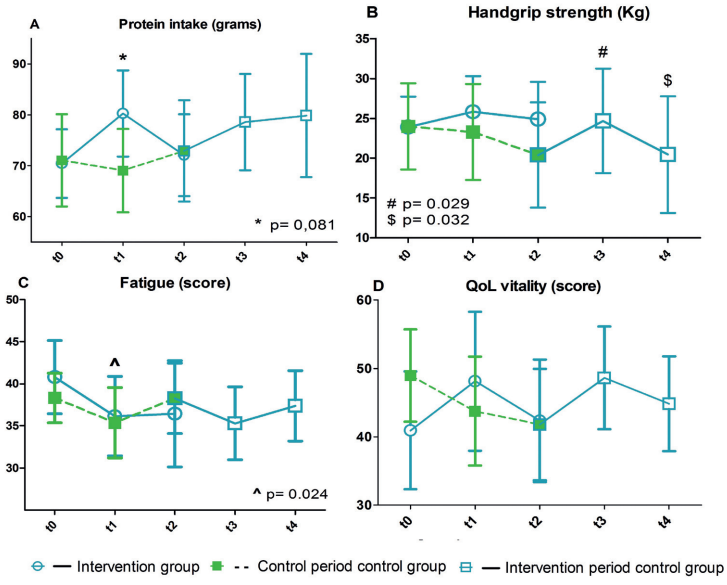

Figure 3. Effect of individually tailored dietary intervention and time comparison between intervention and control group. Data are shown as mean ±95% CI. Study period is the measurement moment; t0: baseline, t1: 3 months, t2: 6 months, t3: 9months, t4: 12 months.

DISCUSSION

This study is unique in being the first explorative RCT to study the effect of an individually tailored dietary intervention on achieving personal goals in MD patients carrying the m.3423A>G mutation. The personal goals of patients were significantly more frequent achieved in the intervention group compared to the control group. Even in this small group of patients we observed a consistent effect on objective and subjective functional outcome parameters: HGS, fatigue and the vitality component of the QoL, ruling out coincidence due to multiple testing. The results are highly relevant and in line with effect of diet counselling in other diseases with high malnutrition incidence. This suggests that a tailored dietary intervention is promising treatment strategy in a currently not curative treatable disorder and specific dietary interventions should be investigated in future.

The majority of the MD patients defined personal goals for weight, fatigue, and HGS. This corresponds well with frequently occurring symptoms in this patient population [30, 31]. In the current study, 57% of the treatment goals were achieved within three months of the dietary intervention. This is in line with a study in obese patients (43% personal goals achieved) [32]. To obtain a successful dietary intervention it is often crucial to adapt behaviour, which is a complex and difficult process [33]. Keeping this in mind, achievement of 57% of the goals in our study can be considered as a positive result. More importantly, these data show that at the individual level it is possible for MD patients to improve their BC and FFM, which is in contrast to other neuromuscular disorders [34].

The patient characteristics at baseline show that malnutrition frequently occurs in this patient category, but that heterogeneity is present. The PG-SGA scores suggest that 55% of the patients in our study population would benefit from a nutritional intervention. Analyses of the goals demonstrated that this nutritional intervention was successful in achieving at least one goal in 97% of patients. This implies that also patients who do not score high for the PG-SGA may benefit from such a strategy.

When analysing the personal goals, a relevant improvement in BC was demonstrated on a group level, however no effect of the intervention on protein intake and body composition was observed. Non-compliance might be an issue here, as well as the relatively small patient population, heterogeneity, the non-standardized intervention, as well as inter-dependency of various variables. The fact that no change in weight at group level was observed might be due to the existence of contradicting goals in the population: losing and gaining weight goals. Concerning protein intake only half of the patients (n=20) were advised to increase their intake and most of them (n=16) actually did. This seems a relevant result, but at the total study population level significance was not achieved, probably due to the low power (p=0.081 for the first three months Figure 2A). Furthermore, some patients had already received dietary advice prior to entering the study and achieved some goals, which made it probably more difficult to realize additional goals.

Multiple factors may underlie our results, including improved nutritional intake and improved nutritional status. There were no differences in activity levels between groups that might explain these results. Generally, it is known that lifestyle changes and focus on healthy eating can make healthy people feel more energetic. This effect seems to apply on the MD population as well.

A significant effect for QoL was only shown for the vitality score and not for the seven other domains for QoL. That this might be due to the effect of personal attention is corroborated by the fact that in the control group QoL improved as well, especially in the first three months of the study.

Our results suggest that the individually tailored dietary intervention was most effective after the first three months and that this effect for most patients did not last. One possible reason for this is that these patients are highly motivated with a good compliance directly after the advice is given, but that this drive decreases afterwards, similar to observations in this setting in other populations [35]. The positive results patients achieved in the first three months were for many not enough motivation to continue with the diet in the same way. This shows how difficult behavioural changes are and that we should support patients in the best possible way. There were no significant differences observed in patient characteristics between patients who achieved more than 50% of their personal goals after three or six months than patients who didn't (see supplement 3).

A limitation of this study was that it, for obvious reasons, could not be conducted in a blinded manner. The fact that the severely malnourished patients were not included was also a drawback because a larger effect of dietary intervention would be expected in these patients. Furthermore, that gastrointestinal complaints could not be analysed on a group level was a limitation, because of the non-continuous nature of this variable. Finally, the fact that there is no gold standard to measure nutritional status was a drawback because we had to categorize our primary outcome variable into many sub variables which led to multiple testing. Therefore, interpretation of the results is more difficult. The meaning of statistical corrected significance in a heterogeneous patient group like this is debatable. For patients achieving individual goals that they choice themselves seems much more relevant than a statistical significant result on a group level.

A strong aspect of this study is the way the personalized health care principles were implemented in an explorative RCT. Although the individually tailored nature of the intervention hindered evaluation on the population level, it was shown that the individually set goals were more frequently achieved after the intervention compared to controls. Also, the fact that goals and interventions were defined by patients in consensus with one dietician is a strong point [36], as well as the use of various validated and well-known scales and assessment tools. The fact that all patients were treated in the same centre is also a strength because there was less variability in treatment approach.

CONCLUSION

This study demonstrates that an individually tailored dietary intervention supports MD patients to achieve personally defined goals, with a beneficial effect on BC, physical functioning and QoL. The effects in this explorative RCT were largely short-lasting but hold promise for more specific RCTs in the future. Gastrointestinal complains could be studied further preferably in a RCT with a single intervention and with a better outcome variable than the Ilikert scale that was used in this study. Nutritional assessment and personalized interventions should be considered in all MD patients. Using personal goals of individual patients is valuable for evaluating the effect of a nutritional intervention.

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AUTHORS' CONTRIBUTIONS

Heidi Zweers was responsible for drafting the manuscript and acquisition of the data. Heidi Zweers and Debbie Smit completed the statistical analysis and interpretation. All authors critically revised the manuscript for important intellectual content. Heidi Zweers, Geert Wanten and Mirian Janssen designed the study protocol. Mirian Janssen was responsible for study supervision.

DISCLOSURES

Heidi Zweers received funding for this study from Vitaflo International Ltd, Liverpool, United Kingdom. Debbie Smit, Susanne Leij, Geert Wanten and Mirian Janssen report no disclosures.

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SUPPLEMENT 1

Detailed individual results from the first 3 months of diet intervention.
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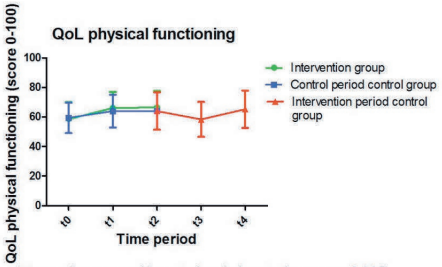
SUPPLEMENT 2

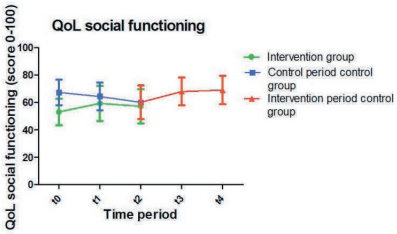
Effect of individually tailored dietary intervention group analyses Quality of Live.

		Intervention group			Control group				
					Control period		Intervention Period		
		T0	T1	T2	T0	T1	T2	T3	T4
Physical functioning		59	66	67	59	64	64	58	65
	upper limit	70	77	78	70	75	77	70	78
	lower limit	47	56	56	49	53	51	47	53
Role functioning physical		40	46	42	37	46	41	45	63
	upper limit	53	56	51	46	55	50	51	76
	lower limit	28	36	33	29	37	37	38	49
Bodily pain		61	66	61	63	60	63	61	62
	upper limit	73	78	73	73	70	72	70	72
	lower limit	48	54	49	53	50	54	52	52
General health		42	43	42	39	42	28	36	34
	upper limit	54	55	54	47	50	36	45	44
	lower limit	30	31	31	31	33	20	27	25
Vitality		41	48	42	49	44	42	49	45
	upper limit	50	58	51	56	52	50	56	52
	lower limit	32	38	33	42	36	34	41	38
Social functioning		53	59	57	67	65	60	68	69
	upper limit	63	72	70	77	75	72	78	77
	lower limit	44	47	45	58	54	48	58	59
Role functioning emotional		51	60	46	63	65	66	63	67
	upper limit	66	72	56	76	78	80	76	79
	lower limit	37	48	36	49	53	37	51	54
Mental health		64	66	70	75	75	70	77	75
	upper limit	73	76	79	82	82	81	84	84
	lower limit	55	57	61	68	68	60	69	67

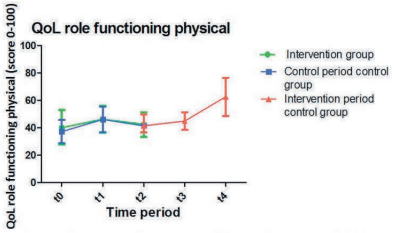
Data are shown as mean +95% CI.



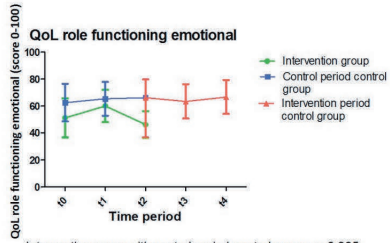
Intervention group with control period control group: p=0.095
Intervention group with control period control group and Intervention period control group: p=0.470
Intervention period control group with control period control group: p=0.807



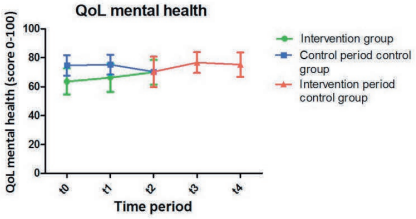
Intervention group with control period control group: p=0.336
Intervention group with control period control group and treatment period control group: p=0.204
Intervention period control group with control period control group: p=0.893



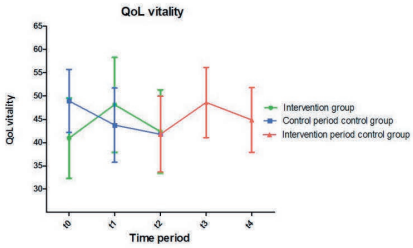
Intervention group with control period control group: p=0.686
Intervention group with control period control group and treatment period control group: p=0.862
Intervention period control group with control period control group: p=0.568



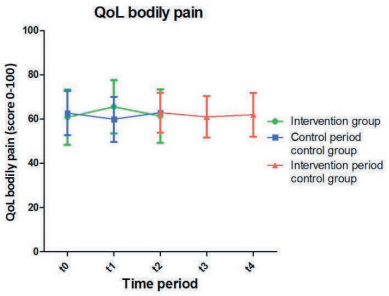
Intervention group with control period control group: p=0.895
Intervention group with control period control group and treatment period control group: p=0.603
Intervention period control group with control period control group: p=0.788



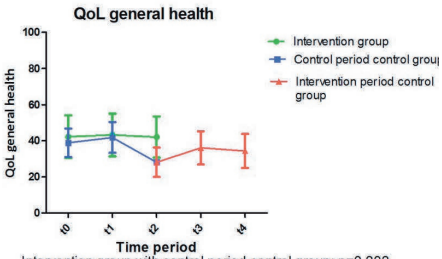
Intervention group with control period control group: p=0.088
Intervention group with control period control group and treatment period control group: p=0.117
Intervention period control group with control period control group: p=0.522



Intervention group with control period control group: p=0.026
Intervention group with control period control group and intervention period control group: p=0.037



Intervention group with control period control group: p=0.696
Intervention group with control period control group and intervention period control group: p=0.736
Intervention period control group with control period control group: p=0.990



Intervention group with control period control group: p=0.202
Intervention group with control period control group and treatment period control group: p=0.054
Intervention period control group with control period control group: p=0.975

SUPPLEMENT 3

Patient characteristics comparison between <50% goals achieved and ≥50% goals achieved after 3 and 6 months diet intervention.

	3 months ≥50% goals achieved (n=28)	3 months <50% gaols achieved (n=10)	6 months ≥50% goals achieved (n=21)	6 months <50% gaols achieved (n=15)
Age (years)	45 ± 12	52 ± 15	46 ± 10	46 ± 17
Gender Female n (%)	23 (82)	8 (80)	16 (76)	13 (87)
NMDAS score	16 ± 9	22 ± 12	17 ± 10	19 ± 10
NMDAS ≥ 20	9 (32)	5 (55)	7 (35)	6 (40)
Heteroplasmy UEC	49 ± 21	48 ± 24	50 (24)	49 (17)
Phenotypes n (%)				
MIDD	15 (54)	5 (50)	11 (52)	9 (60)
Myopathy	11 (39)	5 (50)	8 (38)	6 (40)
MELAS	2 (7)	0	2 (10)	0
Diet before intervention n (%)	18 (64)	7 (70)	13 (62)	10 (67)
Height (m)	1.66 ± 8	1.66 ± 12	1.67 ± 9	1.66 ± 9
Weight (kg)	66 ± 14	73 ± 17	67.2 ± 10	69.7 ± 9
BMI (kg/m^{2})[1]	23.8 ± 4	26.3 ± 6	24.3 ± 4	25 ± 6
BMI< 20 n (%)	3 (11)	1 (10)	1 (5)	3 (20)
BMI> 25 n (%)	10 (36)	6 (60)	9 (43)	6 (40)
PG-SGA score	4.3 ± 3	3.7 ± 3	4 ± 3	4 ± 2
FFMI kg/m^{2}	15.5 ± 2	16.3 ± 2	14.7 ± 2	15.9 ± 3
Fat percentage (9%)	34 ± 5	36 ± 11	35 ± 8	35 ± 6
Fatigue score	39 ± 8	41 ± 9	40 ± 8	40 ± 8
Handgrip strength (kg)	23 ± 9	25 ± 11	24 ± 11	23 ± 9
Exercise METS	1.4 ± 0.2	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
Sleep duration (h:mm)	7:42 ± 1:00	7:43 ± 1:03	7:39 ± 1:05	7:57 ± 0:55
Medication change during intervention n (%)	11 (40)	2 (20)	7 (33)	5 (33)
Smoker n (%)	3 (11)	2 (20)	1 (5)	3 (20)

No differences between groups for all variables

CHAPTER 7

Ketogenic diet for mitochondrial disease: a systematic review on efficacy and safety

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ABSTRACT

Background

No curative therapy for mitochondrial disease (MD) exists, prioritizing supportive treatment for symptom relief. In animal and cell models ketones decrease oxidative stress, increase antioxidants and scavenge free radicals, putting ketogenic diets (KDs) on the list of management options for MD. Furthermore, KDs are well-known, safe and effective treatments for epilepsy, a frequent symptom of MD. This systematic review evaluates efficacy and safety of KD for MD.

Methods

We searched Pubmed, Cochrane, Embase and Cinahl (November 2020) with search terms linked to MD and KD. From the identified records, we excluded studies on Pyruvate Dehydrogenase Complex deficiency. From these eligible reports, cases without a genetically confirmed diagnosis and cases without sufficient data on KD and clinical course were excluded. The remaining studies were included in the qualitative analysis.

Results

Only 20 cases (14 pediatric) from the 694 papers identified met the inclusion criteria (one controlled trial (n = 5), 15 case reports). KD led to seizure control in 7 out of 8 cases and improved muscular symptoms in 3 of 10 individuals. In 4 of 20 cases KD reversed the clinical phenotype (e.g. cardiomyopathy, movement disorder). In 5 adults with mitochondrial DNA deletion(s) related myopathy rhabdomyolysis led to cessation of KD. Three individuals with POLG mutations died while being on KD, however, their survival was not different compared to individuals with POLG mutations without KD.

Conclusion

Data on efficacy and safety of KD for MD is too scarce for general recommendations. KD should be considered in individuals with MD and therapy refractory epilepsy, while it is contraindicated in mitochondrial DNA deletion(s) related myopathy. When considering KD for MD the high rate of adverse effects should be taken into account, but also spectacular improvements in individual cases. KD is a highly individual management option in this fragile patient group and requires an experienced team. To increase knowledge on this -individually- promising management option more (prospective) studies using adequate outcome measures are crucial.

INTRODUCTION

Mitochondrial diseases (MDs) are a heterogenous group of inborn metabolic diseases caused by defects in the genes encoding mitochondrial proteins that are required for ATP production from oxidation of substrates via the tricarboxylic acid cycle and the oxidative phosphorylation (OXPHOS). Underlying pathogenic variants can be found in nuclear or mitochondrial DNA (mtDNA) [1, 2]. Currently more than 295 different disorders are known [2]. While every single disorder is rare, the combined estimated lifetime risk of 235 investigated nuclear encoded autosomal recessive MDs (corrected for the beta-oxidation defects), was calculated 18.74/100,000 based on the global Genome Aggregation Database (gnomAD) equalling e.g. 3,260 affected individuals/17.4 million Dutch inhabitants [3]. Virtual all the extremely heterogeneous symptoms can occur with onset at all ages, but typically tissues with high energy requirements like skeletal/heart muscle and brain are mainly affected. Currently no curative treatment is available, making the supportive management for symptom relief priority.

Given the key role of mitochondria in energy metabolism and the importance of vitamins and co-factors for proper mitochondrial function, nutritional interventions are an integral component of daily management [4-8]. Moreover, nutritional interventions focusing on nutritional status or gastro-intestinal complaints have been shown effective [4, 5].

A ketogenic diet (KD) is a low-carbohydrate high-fat diet that shifts metabolism towards' β-oxidation and ketone body production. Three kind of KDs are defined. The classic KD uses grams of fat: grams of carbohydrate plus protein-ratio (e.g. 4:1 or 3:1) in every meal. Fat can be (mainly) given as medium-chain triglyceride (MCT), this subtype is called MCT-KD. As MCT-fats are converted easier into ketones than longer chain fatty acids, ketosis can be achieved more easily, and more carbohydrates can be consumed. In contrast, the modified Atkins diet (MAD) only restricts carbohydrates (10 - 20 gram per day) without restricting the amount of fat and protein [9-11].

KDs have been proven successful in the treatment of intractable epilepsy and are generally well tolerated and safe [10, 12, 13]. It is thought that KDs exert their positive effect (among others) via stimulation of mitochondrial biogenesis, improvement of mitochondrial function and decrease of oxidative stress [14-17] and therefore have been implemented in some cases with MD and epilepsy [13, 18]. There are also studies suggesting a potential beneficial effect of KD in MD, besides reducing seizures [15, 19]. However, this was mainly studied in patient derived fibroblasts and animal models [9, 15, 20-24]. Of note, while it was previously assumed that the liver provides ketone bodies to the brain, astrocytes itself have shown to be ketogenic cells. This astrocyte ketogenesis might control the survival/death decision of neural cells at least twofold. By scavenging non-esterified fatty acids, the ketogenic pathway could prevent the detrimental actions of these metabolites and their derivatives (e.g. ceramide) on brain structure and function.

Further, by acting directly as pro-survival metabolites, the ketone bodies may preserve neuronal synaptic function and structural stability [25].

When a diet provides only small amounts of glucose, hormones as glucagon inhibit glycolysis and stimulate ketogenesis. These ketone bodies can only be produced in the liver and in astrocytes and provide the mitochondrial OXPHOS with a substrate for energy production. The fatty acid pathway provides 5.7 times more flavin adenine dinucleotide (FADH2) than the glycose pathway and therefore fat has a potential benefit over carbohydrates as an energy substrate in human complex 1 deficiency [26]. However, nicotinamide-adenine-dinucleotide (NADH) is still formed from all substrates and complex 1 is never completely bypassed (Figure 1).

Pyruvate dehydrogenase complex (PDHC) deficiency hampers the conversion of pyruvate to acetyl-CoA and KDs are the pathomechanism based therapy as ketones, converted to acetyl-CoA, bypass the PDHC [27]. Pyruvate Carboxylase deficiency is a contra indication for KD as gluconeogenesis is impaired and affected individuals depend on nutritional glucose.

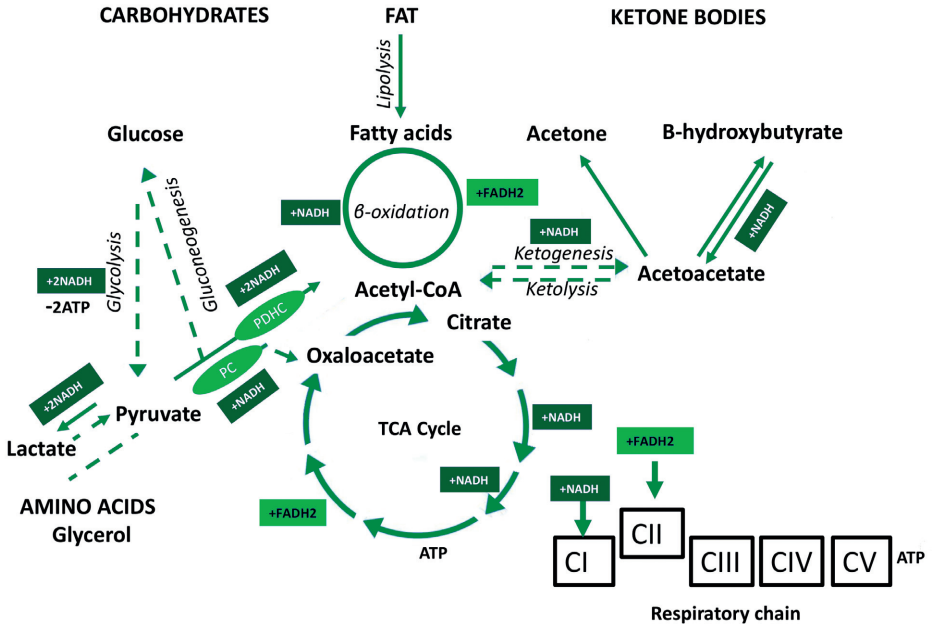

Figure 1. Metabolic pathways of carbohydrates, fat and ketone bodies in energy metabolism

ATP = Adenosine triphosphate, C = respiratory chain complex, PDHC = Pyruvate Dehydrogenase Complex, PC = Pyruvate Carboxylase, TCA Cycle = Tricarboxylic acid cycle also called citric acid cycle.

Taken together, KDs are an interesting management option for MD that needs further evaluation. We here perform a systematic literature review to assess efficacy and safety of KD for MD.

METHODS

Search strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [28]. We identified relevant studies using medical subject headings (MeSH) and text words related to KD and MD (see Supplement 1). The databases searched were: Pubmed, Cochrane, Embase and Cinahl (November 2020) without any search limits. The search strategy for Pubmed was generated together with a specialist librarian and accordingly amended for the other databases.

Study selection

Two authors (HZ, AvW) independently screened and selected the papers using Rayyan® [29]. Eligibility criteria were: cases with MD using a KD and English language. The same authors reviewed full texts of these selected papers independently, according to exclusion criteria. Disagreements were resolved by consensus and discussion with a third author (SBW). Exclusion criteria were: i) cases without genetically proven MD [1, 2], ii) cases with PDHC-deficiency, iii) cases not on KD or without details of the KD composition and iiii) cases without data of effect on clinical phenotype before and under treatment.

KD was defined as any dietary manipulation of fat, carbohydrate and protein aiming to achieve ketosis and included the 'classic' KD, MCT-KD or MAD [9-11]. High fat diets including the low glycaemic index diet are not likely to achieve ketosis and therefore cases treated with these diets were excluded.

Reference lists were reviewed for additional publications.

Outcome measures

The primary outcome was the effect of KD on clinical phenotypes (epilepsy, muscle involvement, tonus dysregulation (muscular hyper- or hypotonia), movement disorders, developmental delay and intellectual disability (DD/ID), other individual signs and symptoms) and the occurrence of adverse events (AEs). The secondary outcome was defined as the effect of KD on MRI findings and laboratory values (e.g. lactic acidosis, liver function test).

Data extraction and quality appraisal

Two authors (HZ, AvW) extracted data and checked the data for completeness. A third author (SBW) checked all articles again to ensure correct interpretation of data. Discrepancies were resolved through discussion and consensus.

We used The Oxford Levels of Evidence 2 [30] scoring to assess study quality as well as The Risk Of Bias In Non-Randomized Studies – of Interventions (ROBINS-I) assessment tool [31]. No studies were suitable for pooling of the results and therefore a narrative analysis is presented.

RESULTS

The search strategy yielded 1149 abstracts (PRISMA flowchart, Figure 2 [32]) of which 17 papers reporting 20 cases were included in the detailed analysis. All data are summarized in Table 1 and Figure 3. Of note, (multi)vitamins and other food supplements were reported in many of the included cases (see Table 1 for details). With exception of one case (TPK1 [33]) clinically not responding to thiamine supplementations, none of the reported vitamins or co-factors were pathomechanism based treatment options and therefore were not taken into account in our analysis.

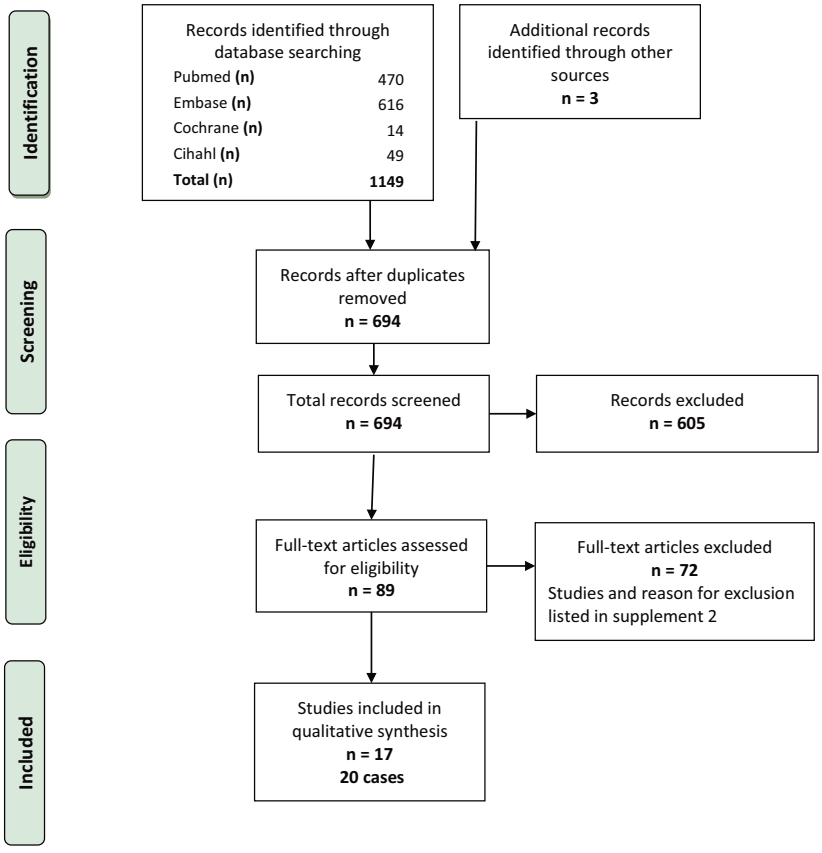

Figure 2. PRISMA flowchart detailing the search strategy.

Figure 3. Summary of positive and negative effects of ketogenic diet in 20 cases with genetically proven mitochondrial disease.

Figure 3 visualises the negative effects and adverse events on the left and the positive effects (on the right) of ketogenic diet in 20 cases with genetically proven mitochondrial disease.
**temporary effect **cases with reported treatment-withdrawal effect B/B = bladder and bowel, CPEO = chronic progressive external ophthalmoplegia , del = deletions, HC = head control, mult = multiple deletions in mitochondrial DNA, MRI = magnetic resonance imaging, mtDNA = mitochondrial DNA, SLE= stroke like episodes, SM = skeletal muscle, TD = tonus dysregulation*

Case characteristics

Of the 20 cases (12 female), 16 had a nuclear DNA and 4 a mtDNA related mutation. Of note 3 of the nuclear variants (TWNK) lead to multiple mtDNA deletions. The ages at start of KD ranged between 0 and 62 years (1 neonate (aged 7 days), 2 infants (9 and 10 months), 11 children (1.3 to 16 years), 6 adults (aged 22 to 62 years). Eight individuals (8/20), were described as having epilepsy. In 10/20 individuals muscle involvement (cardiomyopathy, muscle weakness, exercise intolerance, ptosis) was reported, 7/20 were described having tonus dysregulation, and for 5/20 cases movement disorder(s) (ataxia, dystonia) were reported. In 10/20 cases DD/ID was reported. Other reported symptoms were visual problems, respiratory distress, headaches/migraine, failure to thrive, feeding problems, gastro-intestinal problems, alopecia and hearing loss.

Study quality

The general study quality was low, with 15 case reports (category 4) and one controlled trial with 5 adult participants (category 3b). The risk of bias in the trial was scored as low.

Interventions

Eleven cases followed a classical KD, 8 cases a MAD. For one case the composition of the KD was not detailed, however, this case was included as achievement of ketosis was documented. For a total of 14 cases achievement of ketosis was reported. The total diet duration of all 20 cases together was > 22 patient years, the median diet duration was 4 months (range 4 days -9 years). The main reasons for discontinuation of the diet (n=12) were death (n=3) [34-36] and other AEs (n=7) [37-40]. In one case the daily constraints [41] and in one case the child's craving for carbohydrates and lack of improvement of hearing [42] lead to cessation after 2 years and 4 months, respectively.

Primary outcomes

Effect of KD on epilepsy

In 7/8 cases with uncontrolled epilepsy seizure control was achieved with KD (5 seizure free, 1 reduction of seizures and 1 "stabilisation" of status epilepticus). In 4 of these cases the effect was only temporary (lasting a few weeks to 7 months). In one case no positive effect of the KD on epilepsy was reported, and the KD was stopped after 5 days due to metabolic acidosis.

Effect of KD on other clinical signs and symptoms

Skeletal and heart muscle

In 3/10 cases with muscle involvement KD had a positive effect. This was the return of bladder and bowel control, and the ability to walk with assistance [34], and the complete resolve of chronic progressive ophthalmoplegia (CPEO) and ptosis [41]. In the third case, the 3-year-old individual regained walking abilities with improved lower limb muscle strength after one year of KD and normalisation of septum thickness of hypertrophic cardiomyopathy after 3 years of KD. This effect was sustained at the last follow up at age 11 years (total 8 years of KD) [43]. A negative effect was observed in 6/10 individuals. Five of these six individuals experienced rhabdomyolysis, headache and fatigue leading to cessation of KD after 4 to 11 days [39] and in one case progressive muscular hypotonia with swallowing difficulties was reported going along with a weight loss below the 3rd percentile [38].

Tonus dysregulation

Tonus dysregulation was reported to improve in 3/7 cases, while in one case progressive muscular hypotonia was only reported after the KD was started [38]. In all 3 cases head control improved [33, 44, 45] and one case additionally showed improved stability of truncal tone enabling him to stand with support and to sit independently [33].

Movement disorder

In one case the paroxysmal ophisthotonic dystonia completely resolved for more than 5 years of follow-up [46]. In one individual the ataxia did not improve [47], in the other 3 cases no further details were provided [34, 35, 41].

Developmental delay/Intellectual disability

In 3/10 cases with DD/ID a positive effect of KD was reported (e.g. increased verbal response and abilities, social interaction, improved memory) however no data of formal psychological or developmental testing were presented [33, 34, 45]. The other publications did not provide further details.

Other

An improved oral food intake [33], improved weight gain and growth [43] and hair growth in an individual with alopecia [42] were reported in one case each.

Treatment-withdrawal effect

In 3 cases a treatment withdrawal effect was demonstrated. In the case where KD had resulted in hair growth, this was lost again 6 months after cessation of KD [42]. In the individual where KD led to disappearance of the dystonic-opisthotonic episodes within 5 days for 5 months, these reoccurred within a few days when the KD was stopped and disappeared again 4 weeks after reintroducing KD [46]. In the case where CPEO and ptosis resolved within days and remained absent for 2 years on KD, the ptosis partially reoccurred upon "relaxation" of the diet [41].

Adverse events

In 13/20 individuals AEs were reported (Table 1). In one study [39] all 5 adult participants with mitochondrial myopathy (2 mtDNA single deletion, 3 TWNK/multiple mtDNA deletions) stopped MAD within 4- 11 days because of rhabdomyolysis, headache and tiredness.

Three individuals, all with POLG-related Alpers syndrome, died during KD (at the ages of 46 months [35], 66 months [34] and 16 years [36]) of respiratory failure (n = 2) or paralytic bowel obstruction (n = 1). This was 3 months (n = 2) [35, 36] and 35 months [34] respectively, after presentation.

Other AEs that lead to immediate cessation of the KD were severe lethargy, which occurred after 5 months of KD in one case [40] and lactic acidosis (after 5 days of KD) in one other case [37]. Of note, two individuals in two reports remained on KD despite AEs. In one case because of successful seizure reduction despite a sudden deterioration of visual acuity, ptosis and general weakness after 6 months of KD [47] and in one case the resolve of the movement disorder outweighed the worsening seen on MRI [46]. In one individual [38] who suffered from weight loss, regression and hypotonia the KD was continued for 3.3 months and then weaned to a more conventional feeding regime.

Secondary outcomes

From the 12 cases with reported MRI abnormalities for only 2 cases MRI details were provided after KD initiation. In one case a resumed myelination during KD was observed [45], while in the second the MRI worsened [46]. Interestingly in the latter case, the movement disorder had completely resolved, and the patient was reported to develop age-adequately. In 7 cases lactic acidosis was reported which normalised in one [40], worsened in one [37] and did not change in 2 cases [38, 41]. For the remaining 3 cases no details were reported [43, 44, 47]. Two individuals with POLG mutations had mildly elevated liver function tests before KD initiation [34, 35]. In one case these remained elevated [34], in the other case no details were provided [35]. In one individual a transient, four day long, increase in liver function tests occurred [36].

DISCUSSION

Despite identifying 694 studies using our search strategy, only 20 cases (one controlled trial (n = 5) and 15 case reports) were of sufficient quality for detailed analysis. These data are too scarce to draw firm conclusions regarding efficacy and safety of KD. Future reports on KD for MDs must present a minimum of "common data elements" (in line with the data shown in Table 1) describing the composition of KD as well as the clinical effect including adverse events.

KD is effective for seizure control in MD

KD was highly effective and led to seizure control of therapy refractory seizures in 7 of 8 MD cases, at least temporarily. KD was stopped only after 5 days in the 8th case and it remains elusive if adaptation of the KD would have overcome the occurring lactic acidosis and would have led to seizure control. The number of cases does not allow comparing with the efficacy of KD for intractable seizures of other causes (up to 55%/ 25% becoming seizure free on 4:1 classical KD/ MAD [10]).

KD might be effective for the treatment of other signs and symptoms of MD in individual cases, but is contraindicated in mtDNA deletion(s) related myopathy

In 12/20 cases KD was initiated for other indications than epilepsy. In 5 adults with mitochondrial myopathy KD was stopped due to AEs in all participants [39]. However, a potential long-term benefit cannot be excluded, as the authors report a slight improvement on muscle strength and in 6-minute walking test in three of four patients after 2.5 years of follow up after cessation of KD.

In the remaining 7 pediatric cases, 5 improved clinically [33, 41-43, 46], mainly concerning muscle symptoms. Especially the well reported treatment-withdrawal effect in three cases (start/stop hair growth, resolving/reoccurring ptosis or movement disorder) illustrates the potential for KD in individual management of MD. Of note, in one case

hypertrophic cardiomyopathy was completely resolved on KD and sustained without any additional medication [43]. The authors discuss that ketone bodies may have modulated cardiac metabolism. This is in line with the data suggesting that in heart failure due to metabolic dysfunction fatty acids allow for sufficient energy production while carbohydrates may contribute to declining contractile function. A role for ketones both in signalling as well as an energy source is suspected to underlie this [48].

Safety aspects of KD for MD

AEs occurred 65% of MD cases during KD. This percentage is comparable with studies on KD for PDHC deficiency (13/19 = 68%) [27] or epilepsy with mitochondrial dysfunction (22/34 = 65%) [13, 18]. AEs of KD reported in literature for refractory epilepsy are mainly gastrointestinal complaints rarely leading to discontinuing of the diet, but also lethargy and acidosis have been reported [10, 12].

The 3 children with MD that died (aged 66 -192 months) while being on KD all had POLG-related Alpers disease, an early lethal disorder with a median age at death of 16 (range 1 - 181) months [49]. Hence, their age of death is comparable. Moreover, the median survival after presentation is reported to be 5 (0.5 -181) months without KD [49] and was 3 (n=2) and 35 months, in the cases with KD reviewed here.

Hence, from these limited data it seems unlikely that KD negatively influenced mortality, but is in line with the natural disease course of childhood onset MD.

Practical recommendations

The current guidelines on KD list complex I deficiency as a condition for which KD has been shown reportedly more beneficial when compared to the average response to KD in refractory epilepsy [12, 13, 50] in general. This pathomechanism approach assumes that fatty acids compared to carbohydrates produce more FADH that can enter complex II [13, 19, 50, 51] and hence allows (partial) bypassing of complex I. However, as outlined in the introduction (Figure 1), NADH is still formed from all substrates and complex I is never completely bypassed.

Our study did solely include cases with known genetic background as increasing knowledge from next generation studies shows that complex I deficiency cannot only be seen in MD but also in other genetic diseases especially if measured in muscle specimen of patients with terminal disease [52]. Hence, there is insufficient evidence that KD is more beneficial in mitochondrial complex I related disease than in other MD [12] or even other therapy refractory epilepsies. However, from our results we conclude that KD should be considered in MD patients with therapy resistant epilepsy.

Given the risk of AEs KD should be initiated by a team experienced with both MD and KD. Especially in the first weeks clinical and laboratory controls should be frequent (in

line with the general guideline on KD). From our data we cannot conclude after which duration the efficacy can be judged and we therefore recommend a three months trial of KD, in analogy to KD for intractable epilepsy [53]. Whether classic KD or MAD is superior is unknown and an individual top-down (start KD 4:1) or bottom up approach (start with MAD) should be weighed and discussed with patient and/or parents.

An appraisal for high fat diets

In this context, we would like to mention the high fat diets. The beneficial effects of KD for MDs are probably not only based on ketogenesis and energy expenditure from ketone bodies [23, 54]. Two studies that did not met our inclusion criteria, reporting 4 MD cases with complex I deficiency in muscle without a genetic diagnosis, showed improved maximal workload and muscle force under high fat diet [55, 56]. Another n=1 trial reported a high fat diet improving the endurance in a bicycle test when compared to a high carbohydrate diet in one adult (TMEM126B) [51].

There is further interesting evidence to encourage human studies on high fat diets for MD First, supplementing of complex I deficient human fibroblast cell lines with palmitate resulted in protection from cell death caused by glucose withdrawal presumably based on fatty acid induced stimulation of mitochondrial biogenesis. Second, the study of a mouse model of Harlequin complex I deficient mice established that a high fat diet slowed down disease progression regarding major neurodegenerative symptoms and cerebellar atrophy [57].

More studies on high fat diet in humans reporting the aforementioned common data elements are necessary to draw conclusions. However, it also has to be considered that a high-fat diet could downregulate genes involved in the mitochondrial respiratory chain and could thereby worsen the mitochondrial dysfunction [58].

CONCLUSION

Data on efficacy and safety of KD for MD is too scarce for general recommendations. KD should be considered in individuals with MD and therapy refractory epilepsy, while mtDNA deletion(s) related myopathy is a contraindication (as well as Pyruvate Carboxylase deficiency). KD is a highly individual management option in this fragile patient group and requires an experienced team. To increase knowledge on this -individually- promising management option more (prospective) high quality studies using adequate outcome measures are crucial.

ACKNOWLEDGEMENTS

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AUTHORS' CONTRIBUTIONS

All authors substantially contributed to the design of the study. HZ, AvW, SBW acquired, analysed and interpreted data. HZ and AvW drafted the work, all authors revised it critically for important intellectual content and approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Table 1. Details on genotype, phenotype, diet intervention, safety and efficacy of all 20 cases split into cases with and without epilepsy

Author and year [reference]	Involved Gene	MD subgroup	Age start KD (y)	Gender	Composition KD	Ketosis	KD duration (m)	Reason stop KD	Supplements	Development*	Muscles and movement*	Other signs and symptoms*	Adverse events	Positive effects on
CASES WITH EPILEPSY														
Köse, 2020 [37]	*FBXL4 (AR)*	unclear function	0.8	M	NR	+	0.2 (5d)	AE	B, CoQ, R, T	DD	TD	visual impairment, bowel dysmotility bleeding, dysphagia, FP	metabolic acidosis	NR
O'Byrne 2018 [47]	*MTO1 (AR)*	DNA, RNA & protein synthesis/ RNA metabolism	7	F	CKD 4,75:1 - 2:1	NR	>108	NA	CA, CoQ, D, E, R, T	DD	MW, TD, ataxia	behavioural issues	sudden deterioration during flu-like illness (visual impairment, ptosis, generalised weakness)	E (temporary seizure reduction for 4 y)
Steriade, 2014 [59]	*MT-TL1 (maternal)*	DNA, RNA & protein synthesis/ tRNA	22	F	MAD	NR	>12	NA	ALA, BC, CA, CoQ, CR, D, E, FA	NR	NR	migraine, stroke like episodes		E (seizure free for >1 y), stroke like episodes (resolved)
Joshi 2009 [34]	*POLG (AR)*	DNA, RNA & protein synthesis/ Replication	4.6	F	CKD 4:1	NR	9	AE	NR	DD	MW, gait ataxia	visual impairment	death (at age 66 m)	E (temporary seizure free for 7 m), muscle (bladder/ bowel control regained, walking with assistance regained), development (ability to speak).

Table 1. Continued.

Author and year [reference]	Involved Gene	MD subgroup	Age start KD (y)	Gender	Composition KD	Ketosis	KD duration (m)	Reason stop KD	Supplements	Development*	Muscles and movement*	Other signs and symptoms*	Adverse events	Positive effects on
Spiegler 2011 [35]	*POLG (AR)*	DNA, RNA & protein synthesis/ Replication	3.6	F	CKD	NR	3	AE	NR	DD	ataxia	bowel obstruction, dysphagia	death (at age 46 m)	E (temporary seizure free for a few w)
Koessler 2021 [36]	*POLG (AR)*	DNA, RNA & protein synthesis/ Replication	16	F	CKD 4:1	+	3	AE	CoQ, R, T	NR	NR	migraine	death (at age 16 y)	E (temporary improvement of status epilepticus for 3 w)
Pfeiffer 2020 [44]	*SLC25A12 (AR)*	Substrate/ Carrier	1.8	M	CKD 4:1	NR	>4	NA	NR	DD	TD	NR		E (seizure free for > 4 m, TD (improved head and neck control)
Dahlin 2015 [45], Wibon 2009 [60]	*SLC25A12 (AR)*	Substrate/ Carrier	6	F	CKD 3-4:1	+	>20	NA	NR	DD	TD	NR		E (seizure free for >20 m), TD (improved head and neck control) development, (psychomotor, social interaction), MRI
CASES WITHOUT EPILEPSY														
Della-Marina, 2020 [42]	*BCS1L (AR)*	Assembly, complex II	7	F	MAD	+	4	PD	NR	NR	NR	hearing loss, alopecia, sparse, brittle hair.		**hair growth**
Illsinger, 2020 [46]	*ECHS1 (AR)*	Inhibitors	4	F	MAD 1:1	NR	>60	NA	B, T	NR	dystonia	NR	worsening of MRI	**movement disorder (resolved)**

Table 1. Continued.

Author and year [reference]	Involved Gene	MD subgroup	Age start KD (y)	Gender	Composition KD	Ketosis	KD duration (m)	Reason stop KD	Supplements	Development*	Muscles and movement*	Other signs and symptoms*	Adverse events	Positive effects on
Kotecha, 2019 [38]	*LRPPRC (AR)*	DNA, RNA & protein synthesis/ RNA metabolism	0 (7d)	F	CKD	+	3.3	AE	NR	NR		respiratory distress	progressive hypotonia and regression, weight loss	NR
Ahola, 2016 [39]	*mtDNA single del (maternal)*	mtDNA single deletion	62	F	MAD	+	0.1 (4d)	AE	NR	NR	MW, EI, ptosis	NR	RM, headache, tiredness	NR
Ahola, 2016 [39]	*mtDNA single del (maternal)*	mtDNA single deletion	36	F	MAD	+	0.3 (8d)	AE	NR	NR	MW, EI, ptosis	NR	RM, headache, tiredness	NR
Deberles, 2020 [43]	*MT-TW (maternal)*	t-RNA	3	F	CKD 3:1	+	>96	NA	C, CA, E, R, T	DD	MW, CM	FTT		muscle (regained walking, improved limb muscle strength), cardiomyopathy (resolved), weight gain, growth
Laugel 2007 [41]	*NDUFV1 (AR)*	OXPHOS enzymes/ complex I	0.8	M	CKD 3:1	+	24	PD	CoQ, R	DD	MW, TD, ptosis, ataxia, pyramidal signs	vomiting, hyperpnea, strabismus		**CPEO, ptosis (resolved)**
Huang 2017 [40]	*SUCLA2 (AR)*	DNA, RNA & protein synthesis/ Nucleotides	1.3	M	CKD 3:1	+	5	AE	C, CA, CoQ, E, R, T	DD	MW, TD ptosis, hyper-reflexia	hearing loss, FTT, FP, GER, constipation	severe lethargy	lactate (normalised)

Table 1. Continued.

Author and year [reference]	Involved Gene	MD subgroup	Age start KD (y)	Gender	Composition KD	Ketosis	KD duration (m)	Reason stop KD	Supplements	Development*	Muscles and movement*	Other signs and symptoms*	Adverse events	Positive effects on
Fraser 2014 [33]	*TPK1 (AR)*	Cofactors/ Thiamine	1.7	M	CKD 3:1	+	>9	NA	ALA, B, N, T	DD	TD	FP		TD (improved head and neck control, truncal tone stability), development (increased verbal response and social interaction), food intake
Ahola, 2016 [39]	*TWNK (AR), mult del (maternal)*	DNA, RNA & protein synthesis/ Replication	54	M	MAD	+	0.3 (9d)	AE	NR	NR	MW, EI, ptosis	NR	RM, headache, tiredness	NR
Ahola, 2016 [39]	*TWNK (AR), mult del (maternal)*	DNA, RNA & protein synthesis/ Replication	52	M	MAD	+	0.3 (8d)	AE	NR	NR	MW, EI, ptosis	NR	RM, headache, tiredness	NR
Ahola, 2016 [39]	*TWNK (AR), mult del (maternal)*	DNA, RNA & protein synthesis/ Replication	40	m	MAD	+	0.4 (11 d)	AE	NR	NR	MW, EI, ptosis	NR	RM, headache, tiredness	NR

*AE = adverse event, ALA = alpha lipoic acid, AR = autosomal recessive, B = biotin, BC = vitamin B complex, C = Vitamin C, CA = carnitine, CR = creatine, CKD = classical ketogenic diet, CM = Cardio Myopathy, CoQ = idebenone or coenzyme Q10, CPEO = chronic progressive external ophthalmoplegia, d = days, DD = developmental delay, E = epilepsy, F = female, EI = exercise intolerance, FA = folic acid, FFT = failure to thrive, FP = feeding problems, GER = gastroesophageal reflux, ID = intellectual disability, KD = ketogenic diet, m = months M = male, MAD = modified atkins diet, MRI = magnetic resonance imaging, MW = muscle weakness, N = niacin, NA = not applicable, NR = not reported, PD = parental decision, R = riboflavin, RM = rhabdomyolysis, T = thiamin, TD = tonus dysregulation, w = weeks, y = years, *= clinical findings before start KD. **in bold**: cases with reported treatment-withdrawal effect*

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SUPPLEMENT 1

Search strategy

Databases

Four databases were searched

1. Pubmed
2. Cochrane
3. Embase (Ovid)
4. Cinahl

A combination was used of:

- Mesh terms (Medical Subject Headings) from Pubmed, Cochrane and Cinahl or Emtree terms from Embase
- Text-word search in title, abstract and author keywords.

Entry terms

1. "Mitochondrial Diseases"[Mesh]
2. alpers' OR alpers OR huttenlocher
3. complex deficienc*
4. complex 1 OR complex I OR complex 2 OR complex II OR complex 3 OR complex III OR complex 4 OR complex IV OR complex 5 OR complex V
5. chronic progressive external ophthalmoplegia OR CPEO
6. Friedreich Ataxia
7. Kearns Sayre
8. Leigh
9. Leber hereditary optic neuropathy OR LHON
10. mitochondrial encephalomyopathy lactic acidosis stroke-like episode* OR MELAS
11. myoclonic epilepsy ragged-red fibres OR MERRF
12. maternally inherited diabetes deafness OR MIDD
13. maternally inherited Leigh syndrome OR MILS
14. mitochondrial
15. myoneurogenic gastrointestinal encephalopathy OR MNGIE
16. mtDNA
17. Neuropathy ataxia retinitis pigmentosa ptosis OR NARP
18. Oxidative Phosphorylation Deficienc* OR OXPHOS
19. Pearson
20. POLG
21. Respiratory Chain Deficienc*
22. 1 or 2 or 3 or 4 or 6 or 6 or 7 or 8 or 9 or 10 or 11 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21

23. "Diet, Ketogenic"[Mesh]
24. Ketogenic*
25. Modified Atkins
26. 23 or 24 or 25
27. 22 and 26

For Embase the following publications types were excluded: books or chapter or conference abstract or editorial or note or tombstone

Syntax of Mesh/Emtree terms per database.

Entry nr	Pubmed	Cochrane	Embase (OVID)	Cinahl
1	"Mitochondrial Diseases"[Mesh]	MeSH descriptor: [Mitochondrial Diseases] explode all trees	exp "disorders of mitochondrial functions"/	(MH "Mitochondrial Diseases+")
23	"Diet, Ketogenic"[Mesh]	MeSH descriptor: [Diet, Ketogenic] explode all trees	exp ketogenic diet/ exp modified Atkins diet/	(MH "Ketogenic Diet")

Syntax of title, abstract and author keyword per database.

Entry nr	Pubmed	Cochrane	Embase (OVID)	Cinahl
2-21, 24, 25	[tiab]	:ti,ab,kw	.ti,ab,kw.	ti ab

Note: High fat was left out as search term, as the focus is on ketogenic diet.

SUPPLEMENT 2

Excluded studies.

Study / Year	Reason for exclusion
Barzegar 2007 [1]	No genetic mitochondrial diagnose
Ait-El-Mkadem 2017[2]	Unknown diet composition
Al Madhoun 2019 [3]	Unknown diet composition
Banka, 2014[4]	Unknown diet composition
Bernard 2008 [5]	Wrong population: not on ketogenic diet
Bjorkman 2015 [6]	No clear or minimal outcome data
Brivet 2003 [7]	High fat diet
Cardenas 2010 [8]	Unknown diet composition
Emperador 2019 [9]	Wrong study type: cell study
Fahrner 2016 [10]	No genetic mitochondrial diagnose
Friederich 2020 [11]	No clear or minimal outcome data
Fukao 2019 [12]	Wrong population
Garcia-Cazorla 2005 [13]	No clear or minimal outcome data
Grazina 2007[14]	Unknown diet composition
Gropman 2018 [15]	Letter to the editor
Guilliams 2013 [16]	Letter to the editor
Han 2017 [17]	No clear or minimal outcome data
Habarou 2017[18]	Unknown diet composition
Hasan-Olive 2019 [19]	Wrong study type: animal study
Hildick-Smith 2013 [20]	No clear or minimal outcome data
Hussain 2016 [21]	Wrong population
Joshi 2016 [22]	No genetic mitochondrial diagnose
Jung 2012 [23]	No genetic mitochondrial diagnose
Kang 2006 [24]	No genetic mitochondrial diagnose
Kang 2007 [25]	No genetic mitochondrial diagnose
Khan 2012 [26]	Unknown diet composition
Kim 2012 [27]	No genetic mitochondrial diagnose
Klepper 2003 [28]	Not written in English
Klepper 2004 [29]	Not written in English
Kwong 2019 [30]	No genetic mitochondrial diagnose
Lee 2008 [31]	No genetic mitochondrial diagnose
Lee 2010 [32]	No genetic mitochondrial diagnose
Lee 2016 [33]	No clear or minimal outcome data
Lee 2019 [34]	No genetic mitochondrial diagnose
Leung 1998 [35]	No genetic mitochondrial diagnose
Liu 2019 [36]	Wrong population
Malojcic 2004 [37]	No genetic mitochondrial diagnose
Marchio 2016 [38]	abstract (conference published)

Excluded studies (continued).

Study / Year	Reason for exclusion
Martikainen 2012 [39]	Low glycemic index diet
Na 2020 [40]	No genetic mitochondrial diagnose
Nass 2019 [41]	No clear or minimal outcome data
Nathan 2019 [42]	Wrong population
Ngoh 2016 [43]	Unknown diet composition
Nishioka 2018 [44]	No clear or minimal outcome data
Nizon 2014 [45]	High fat diet
Nolan 2019 [46]	No clear or minimal outcome data
O'Connor 2014 [47]	No genetic mitochondrial diagnose
Oonthonpan 2019 [48]	Same case as Brivet 2003; high fat diet
Panetta 2004 [49]	Unknown diet composition
Pronicki 2017 [50]	Unknown diet composition
Punzi 2008 [51]	Unknown diet composition
Salman 2017 [52]	Wrong population: Glut 1
Schmid 2019 [53]	No genetic mitochondrial diagnose
Seaver 2018 [54]	Unknown diet composition
Seo 2010 [55]	No genetic mitochondrial diagnose
Soler-Alfonso 2019 [56]	Unknown diet composition
Sort 2013 [57]	No genetic mitochondrial diagnose
Stowe 2018[58]	Unknown diet composition
Storoni 2019 [59]	Review, no human cases
Sutton 2010 [60]	Wrong population: secondary mitochondrial dysfunction
Taban 2006 [61]	Wrong population: PDHC deficiency
Theunissen 2017 [62]	High fat diet
Vasta 2012 [63]	No genetic mitochondrial diagnose
Villeneuve 2017 [64]	No genetic mitochondrial diagnose
Williams 2012 [65]	No clear or minimal outcome data
Yadav 2019 [66]	No genetic mitochondrial diagnose
Yamanaka 1987 [67]	No genetic mitochondrial diagnose
Yilmaz 2010 [68]	No genetic mitochondrial diagnose
Yoon 2014 [69]	No genetic mitochondrial diagnose
You 2009 [70]	No clear or minimal outcome data
Zhang 2018 [71]	No clear or minimal outcome data
Zupec-Kania 2013 [72]	Review, no human cases

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CHAPTER 8

General Discussion

GENERAL DISCUSSION

The general discussion addresses the aims as defined in the introduction section and reflects on the main findings, implications and future perspectives.

The tree aims as defined in the introduction were:

1. To gain knowledge on the nutritional status and its determinants in adult MD patients.
2. To define the optimal strategy for nutritional assessment in adult MD patients.
3. To provide evidence for individually tailored nutritional interventions in MD patients.

MAIN FINDINGS

Table 1 provides an overview of the aims, main findings and limitations of the studies presented in this thesis.

NUTRITIONAL STATUS OF ADULT MD PATIENTS AND ITS DETERMINANTS

Chapter 2 focused on identifying symptoms that lead to a decreased BMI and increased risk for malnutrition in adult MD patients. Gastrointestinal problems (86%), especially constipation (69%), bloating (51%), flatulence (40%) and mild dysphagia (33%), are very common in carriers of the m.3243A>G mutation. The severity of gastrointestinal problems as well as overall disease severity was associated with an increased risk for malnutrition in the form of a low BMI. There was however no relevant correlation between gastrointestinal problems and BMI, indicating that probably other factors contribute to the risk of malnutrition.

The results support the hypothesis that severely affected patient have a higher risk for malnutrition. Apabhai et al [1] studied a similar MD population in the UK (Newcastle): in contrast these authors found that almost half of the patients were overweight, similar to healthy controls. While **chapter 2** showed a significantly lower BMI in patients compared to controls. Still around one third of these patients had a BMI>25. Therefore, both under- and overweight seems to be a problem in this population. It should be realized here that also patients with normal or high BMI may still be at risk for malnutrition because of an inadequate diet, with altered body composition (low muscle mass and high fat mass) and low functioning.

In **chapter 3** the food intake of the MD patients was evaluated in more detail. Most MD patients had an inadequate diet. Specifically, intake of protein, calcium, dairy products, and fluids were low although interindividual differences were high. Overall, maintaining a healthy

diet seems as difficult for MD patients as it is for the general population: recommendations for fibre, sugars, saturated fat, and vitamin D intake were not met in either group. **Chapter 4** studied all three domains of the malnutrition definition: (protein) intake, body composition and functioning. A lower protein intake could not be established in MD patients when compared with healthy controls ($P = 0.07$). However, relative to their protein needs, more MD patients had a protein intake that was too low (68%) as compared to healthy controls (12%). Two other studies by Hou et al [2] and Aubry et a [3] reported a lower muscle mass in MD patients which could only be confirmed in part. MD patients in our study had a low muscle mass compared to reference data but the controls also had a low muscle mass and therefore any differences between MD patients and controls could not be demonstrated.

The high prevalence of malnutrition in MD patients in our study (73%) confirms previous work [3]. According to the GLIM criteria, 46% of MD patients were malnourished, whereas 43% according to the PG-SGA criteria. This seems a consistent result, however, there was a low overlap in malnutrition between the two methods. A better comparability was seen between sarcopenia and malnutrition since all patients diagnosed with sarcopenia were also classified as malnourished. This low comparability underlines the challenges of diagnosing malnutrition and underlines the conclusions of Aubry et al. (2017) [3] that nutritional assessment should be part of patient care in all adult MD patients.

THE OPTIMAL METHOD FOR NUTRITIONAL ASSESSMENT IN ADULT MD PATIENTS

Measurements of Nutritional Assessment should always comprise tree domains:

1. Food intake and requirements
2. Body composition
3. Functional parameters

Chapter 5 focused on domain 1, i.e. energy requirement. The conclusion is that total energy expenditure is lower in MD patients compared to the recommendations for healthy adults, most probably because of their lower physical activity. In MD patients six predictive equations for resting energy expenditure provide a reliable alternative for Indirect calorimetry, with an accuracy in the range of 71-76%. As Physical Activity Levels (PAL) are highly variable and are not reliably estimated by the patients themselves, measurement of PAL using accelerometery is recommended in this population.

In **chapter 4** the second domain i.e. body composition was evaluated and the diagnostic accuracy of BIA versus DXA measurements in adult MD was tested. The conclusion was that for measuring body composition, DXA should be preferably used instead of BIA because of the higher accuracy. If DXA is not available, waist circumference is the preferred additional

method besides BIA to assess the risk for obesity and metabolic syndrome in this population because BIA has a low sensitivity and specificity for diagnosing a high fat percentage.

In **chapter 4** multiple functional tests from the third domain were executed: handgrip strength, 30 sec sit to stand test, 6 minute walking test and the 6 minute mastication test. The choice for these tests were based on the expertise of the physiotherapist of the multidisciplinary mitochondrial expertise team from the Radboudumc. In both the GLIM consensus for diagnoses of malnutrition [4] and in the sarcopenia consensus [5] handgrip strength is included which is easy to perform by patients, besides also dietitians can include this in their consultation with minimal additional effort or costs. The handgrip strength test provides valuable data on functioning, however reference values and cutoff points are unclear. For instance, in the Sarcopenia consensus cutoff points are used based on data provided by Dodds et al [6] for 70-year-old patients. This makes sense because sarcopenia is mainly a muscle disorder in elderly. However, chronically ill patients, including the MD population, frequently suffer from sarcopenia as well [2,7,8]. Since the mean age of the MD population is between 40-45 years, it is incorrect to use the cutoff point for 70-year-old patients without further evidence. In **chapter 4** is demonstrated that indeed sarcopenia gets underdiagnosed when the mentioned consensus cutoff point are used. Therefore, the recommendation is to use the age-specific cutoff point of the Dodds reference [6].

In **chapter 4** the measurements tools PG-SGA [9] and consensus definitions for Malnutrition (GLIM) [4] to diagnose malnutrition and sarcopenia consensus to diagnose sarcopenia [18] were applied to the adult MD population.

The PG-SGA has been validated in oncology patients and it is suggested to be suitable for other patient categories [10]. The MD patient, however, in contrast to cancer patients in general does not suffer from inflammation. Another drawback of the PG-SGA is that as found in **chapter 6,** the improved nutritional status after diet intervention is not reflected in an improved PG-SGA score. This could be explained by the fact that gastrointestinal symptoms are frequently seen in MD patients, as described in **chapter 2**, which comprise a substantial part of the PG-SGA score. For example, the individual diet intervention could decrease the severity of constipation, but the patient still received 1 point for constipation in the PG-SGA because severity of symptoms is not reflected by a lower PG-SGA score. Moreover the "functioning section" of the PG-SGA contains a question about lowering of daily activity which is not suitable for a chronic patient population. Apart from these drawbacks, the use of the PG-SGA is supported by the positive fact that it is a patient generated instrument that includes all 3 domains of nutritional assessment and takes little effort to measure. In the Radboudumc the PG-SGA is integrated in the electronic patient file (EPIC and mijnRadboud) and data is therefore easy available.

The GLIM criteria for malnutrition are categorized into two domains. One is the phenotypic criterium on body composition. The other domain comprises the etiologic

criterium. To meet this criterium, patients need to have either a low nutritional intake and/or inflammation should be present. Unfortunately, a "functioning domain" is not part of this consensus. The GLIM criteria have not specifically been designed for the analysis of a chronic patient category.

The sarcopenia consensus on the other hand is more suitable for chronic patients, yet sarcopenia shows overlap with malnutrition. Also, while both nutrition and mitochondrial dysfunction can play a role here [179] sarcopenia is a muscle disorder that mainly occurs in elderly. Therefore, the consensus only contains two domains: body composition and functioning. For sarcopenia diagnosis, body composition (muscle mass) has to be measured (different from the PG-SGA or GLIM criteria). Although this measurement is more invasive, body composition is crucial for the MD patient population because many patients with normal or even high BMI still have a low muscle mass, which may easily remain undetected if not actually measured [3]. In the sarcopenia consensus also functioning has to be measured, using either hand grip strength or the sit-to-stand tests. As national expertise centre for mitochondrial disease, the Radboudumc has experience with both of these non-invasive tests. The sit to stand test has additional advantages in MD patients because of the endurance component, which is highly relevant in patients with a muscular energy deficit.

The results of **chapter 4** are in line with the results of Aubry et al [3] who performed nutritional assessment on al domains in 11 MD patients and 15 controls. These authors concluded that all patients were malnourished according to the definition by the European Society of Clinical Nutrition and Metabolism (ESPEN), but none were malnourished according to the screening tool (nutritional risk score NRS-2002). Thus, the latter tool seems less sensitive in chronically ill outpatients.

The overall conclusion is that all available tools to diagnose malnutrition in adult MD patients have their advantages and drawbacks, without one being optimal in this setting. Nutritional Assessment of adult MD patients should always comprise the tree domains (intake and requirements, body composition and functioning). For energy requirements accelerometery is recommended. For body composition DXA is recommended and if that is not possible BIA in combination with a waist circumference is considered second best. For functioning, handgrip strength using age appropriate cutoff points of the Dodds reference [6] or the sit to stand test, are suitable non-invasive test.

INDIVIDUALLY TAILORED NUTRITIONAL INTERVENTIONS IN MD PATIENTS

Part 2 of this thesis focuses on the evidence to support nutritional interventions. The conclusion is that an individually tailored dietary intervention is promising to achieve personalized goals of patients with MD, especially with regard to body composition,

handgrip strength and gastrointestinal complaints. The intervention also improves quality of life and decreases fatigue on the short term.

Data on efficacy and safety of ketogenic diet for MD is too scarce for general recommendations. KD should be considered in individuals with MD and therapy refractory epilepsy, while mtDNA deletion(s) related myopathy is a contraindication. Ketogenic diet is a highly individual management option in this fragile patient group and requires an experienced team. To increase knowledge on this -individually- promising management option more (prospective) high quality studies using adequate outcome measures are crucial.

REFLECTION

Strengths

A strength of this thesis is that all research questions were driven from 20 years of patient care and therefore the relevance to the metabolic dietetic field seems guaranteed. In addition, the results of these studies were beneficial for patients, since all the recommendations have been implemented as part of the personalized patientcare in the Radboudumc national expertise centre for mitochondrial disease (RCMM).

The possibility to perform these studies with the help of the relatively large patient population of the RCMM, and especially the genetically homogeneous cohort of m.3243A>G carriers, was crucial in this respect.

Another strength of this thesis were the selected study methods. This especially concerns the matched-control design of the DYNAMO study, in which the gold standard (DXA) was used to assess body composition. The same applies for the innovative strategy to calculate total energy expenditure by means of accelerometery. Finally, the fact that this thesis includes a randomized controlled trial and a systematic review in a field where until now only case reports were available to guide dietitians practises is of value.

Limitations

The relatively small sample size and the heterogeneity of the patients remain inevitable limitations. The provision of data on nutritional intake is also a weakness in all studies because both under- and over-reporting nutritional intakes probably occurred.

Chapter 6 is an explorative randomized controlled trial on individually tailored nutritional interventions. This was a very challenging study with numerous confounders, ending up in a combination between an RCT with 39 subjects and 39 n=1 trials. We strongly believe that for this heterogeneous patient population individually tailored nutritional advise will result in the best possible care, but the evidence to support this view is very difficult to

provide. For instance, the lack of a gold standard to diagnose malnutrition is a handicap, since testing all the separate items of nutritional assessment caused statistical issues related to multiple testing. Despite these limitations, the DINAMITE study yielded many relevant data and clinical insights.

In **chapter 4** the DYNAMO study**,** has a limitation regarding the validity of the DXA measurements which may have been affected by the use of 2 scanners on two locations: Although these scanners were from the same brand, they were different models and calibration was only possible for bone density, but not for body composition.

IMPLICATIONS AND FUTURE PERSPECTIVES

The nutritional implications and recommendations based on the results of this thesis are summarised in Figure 1. This summary was published as a poster on the SSIEM congress in Rotterdam 2019 [11].

Research agenda

- A prospective intervention study to investigate whether a higher protein intake may improve functioning in MD.
- A RCT with a standardized diet intervention (fodmap diet [12]) in MD patients with gastrointestinal complaints.
- KETOMY study: Safety, efficacy and feasibility of ketogenic diet in mitochondrial myopathy. This is a pilot study in 20 patients with genetically proven mitochondrial myopathy started in October 2020.

GENERAL CONCLUSION

Malnutrition is very common in MD, even in patients with a normal or high BMI. Unfortunately, usual screening tools lack sensitivity to detect malnutrition in this chronically ill patient group. All adult patients with MD should receive full nutritional assessment of all three domains 1) food intake and requirements; 2) body composition; 3) functional parameters. This Nutritional assessment should include adequate interviews checking for gastrointestinal complaints, accelerometry, assessment of body composition (e.g. DXA or BIA with waist circumference) and functional testing (e.g. handgrip strength measurements and/or 30 second sit to stand test). Nutritional interventions should be tailored in a personalized manner and based on the nutritional assessment outcomes. Nutritional interventions can be helpful for MD patients to achieve personal goals in body composition, functioning and decreasing fatigue.

Table 1. Overview of aims, main findings and limitations.

Part	Chapter	Aim (s)	Main findings	Comments/limitations
1	2	Exploring frequency and severity of dysphagia and gastrointestinal problems in carriers of the m.3243A>G mutation. Identification of symptoms that lead to decreased BMI and increased risk for malnutrition.	Dysphagia and gastrointestinal problems, especially constipation, are common symptoms in the total m.3243A>G carriers cohort and are not related to heteroplasmy levels in UEC or disease severity. The severity of gastrointestinal problems as well as overall disease severity is associated with an increased risk for malnutrition.	Risk for malnutrition scored with MUST that is not designed for chronic malnutrition. BMI was used, which provides little information on body composition.
	3	Investigate whether patients with MD have an insufficient or unbalanced food intake and to establish which nutrients and product groups are particularly compromised in this patient group.	Many patients with MD have an inadequate diet. Specifically, intake of protein, calcium, dairy products, and fluids were low. Interindividual differences are high.	The weakness of this study is the use of nutrition diaries this may lead to bias in the results. Furthermore, the data collection method used in the healthy controls is a different method (2-day recall).
	4	Explore associations between physical functioning, body composition and protein intake of patients with MD. Assessing the prevalence of malnutrition and sarcopenia in MD using various nutrition assessment tools. Test the diagnostic accuracy of bioelectrical impedance analysis (BIA) to determine body composition in MD patients.	Muscle strength is related to body composition and protein intake in MD patients. 73% of MD patient were malnourished, and 14-27% were classified as sarcopenic. BIA overestimates fat-free mass compared with DXA.	Lower muscle mass in MD patients compared to controls could not be confirmed and that was mainly because unexpectedly the healthy controls had also a low fat free mass compared to reference. The use off 2 different DXA scanners.

Table 1. Continued.

Part	Chapter	Aim (s)	Main findings	Comments/limitations
	5	To identify the optimal method to estimate total energy expenditure in MD patients.	Total energy expenditure is lower in MD patients than the recommendations for healthy adults because of their lower physical activity. In MD patients, 6 prediction equations for resting energy expenditure provide a reliable alternative for Indirect Calorimetry, with an accuracy of 71%-76%. The patients' own estimations for physical activity levels proved not to be a suitable alternative to accelerometery.	The use of BIA data instead of DXA. BIA is known to overestimate FFM compared with DXA in MD patients (chapter 4). The use of the healthy energy recommendations as a control for total energy requirements, instead of using a healthy control group that had the same accelerometry + Indirect Calorimetry measurements.
2	6	To evaluate the effect of an individually tailored dietary intervention on personalized goals, body composition, functioning, and quality of life in adult patients with MD due to the m.3243A>G mutation.	After 3 months of dietary intervention, 57% of personalized goals were achieved. Most effective goals were body composition, handgrip strength, and gastrointestinal complaints. Consistent effect on functioning included improved handgrip strength, vitality, and fatigue score. Effects did not seem to last after 3 months.	The study could not be conducted in a blinded manner. Gastrointestinal complaints could not be analysed on a group level. That there is no gold standard to measure nutritional status was a drawback because it led to multiple testing. The choice of non-standardized intervention is both a strength and a limitation of this study. It makes interpretation of results more difficult.

Table 1. Continued.

Part	Chapter	Aim (s)	Main findings	Comments/limitations
	7	To review the evidence on the efficacy and safety of ketogenic diet in MD.	From 694 papers only 20 cases met the inclusion criteria. KD led to seizure control in 7 out of 8 cases. In 3 of 10 individuals improvement of muscular symptoms was reported, in 4 of 20 cases a reversal of the clinical phenotype (e.g. cardiomyopathy, ptosis, movement disorder and alopecia) was seen. In 12 of 20 individuals the KD was discontinued mainly because of adverse events. In 5 adults with mitochondrial DNA deletion (s) related myopathy rhabdomyolysis led to cessation of KD. Three individuals with *POLG* mutations died while being on KD.	Data on efficacy and safety of KD for MD is too scarce for general recommendations. KD is effective for seizure control in MD. KD might be effective for the treatment of other signs and symptoms of MD in individual cases. The high rate of adverse effects including death seem to be attributable to the progressive course of MD. Mitochondrial DNA deletion (s) related mitochondrial myopathy seems to be a contraindication for KD. More (prospective) high quality studies using adequate outcome measures are urgently required.

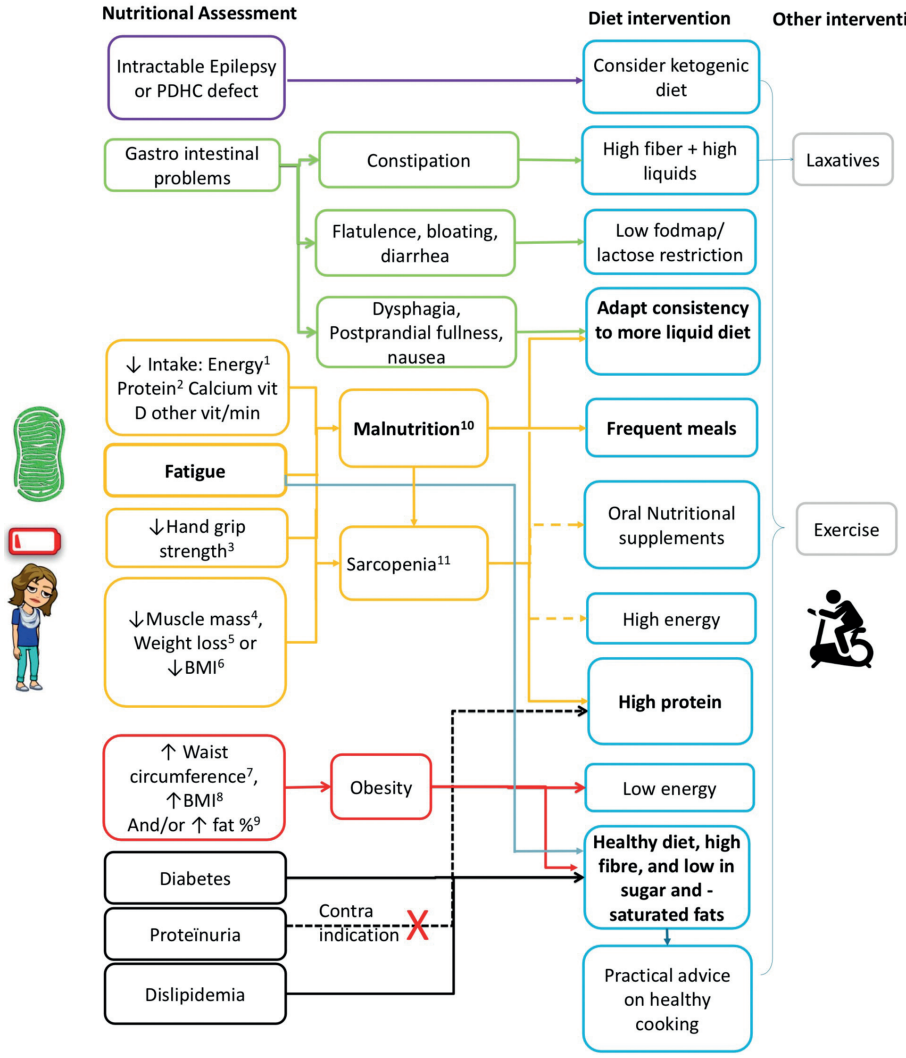

Figure 1. Summary of nutritional implications and recommendations based on the results of this thesis.

1) low energy intake < 90% individual needs = calculated REE + measured PAL (with actometre) 2) low protein intake <1,2 gram/kg ideal bodyweight 3) low handgrip according to (Dodds 2014) 4) low muscle mass; preferable measured by DXA = SMI < 7 kg/m² for men and < 5,5 kg/m² for women or FFMI measured by BIA < 15 kg/m² for women and <17 kg/m² for men (GLIM criteria 2018) 5) weight loss = > 5% in 6 months or > 10% > 6 months (GLIM criteria 2018) 6) low BMI =< 20 kg/m² (GLIM criteria 2018) 7) high waist circumference = men ≥ 94 cm women ≥ 80 cm (WHO 2008) 8) high BMI= >30 kg/m² (WHO 2000) 9) high fat percentage = >30 % for women and >25 % for men (Okorodudu 2010) 10) malnutrition = according to GLIM 2018 = low muscle mass[4], low BMI[6] or weight loss[5] + low nutritional intake[1,2] or malnutrition according to PG SGA 11) sarcopenia= consensus 2018 = low Handgrip Strenght[3] + low muscle mass[4]

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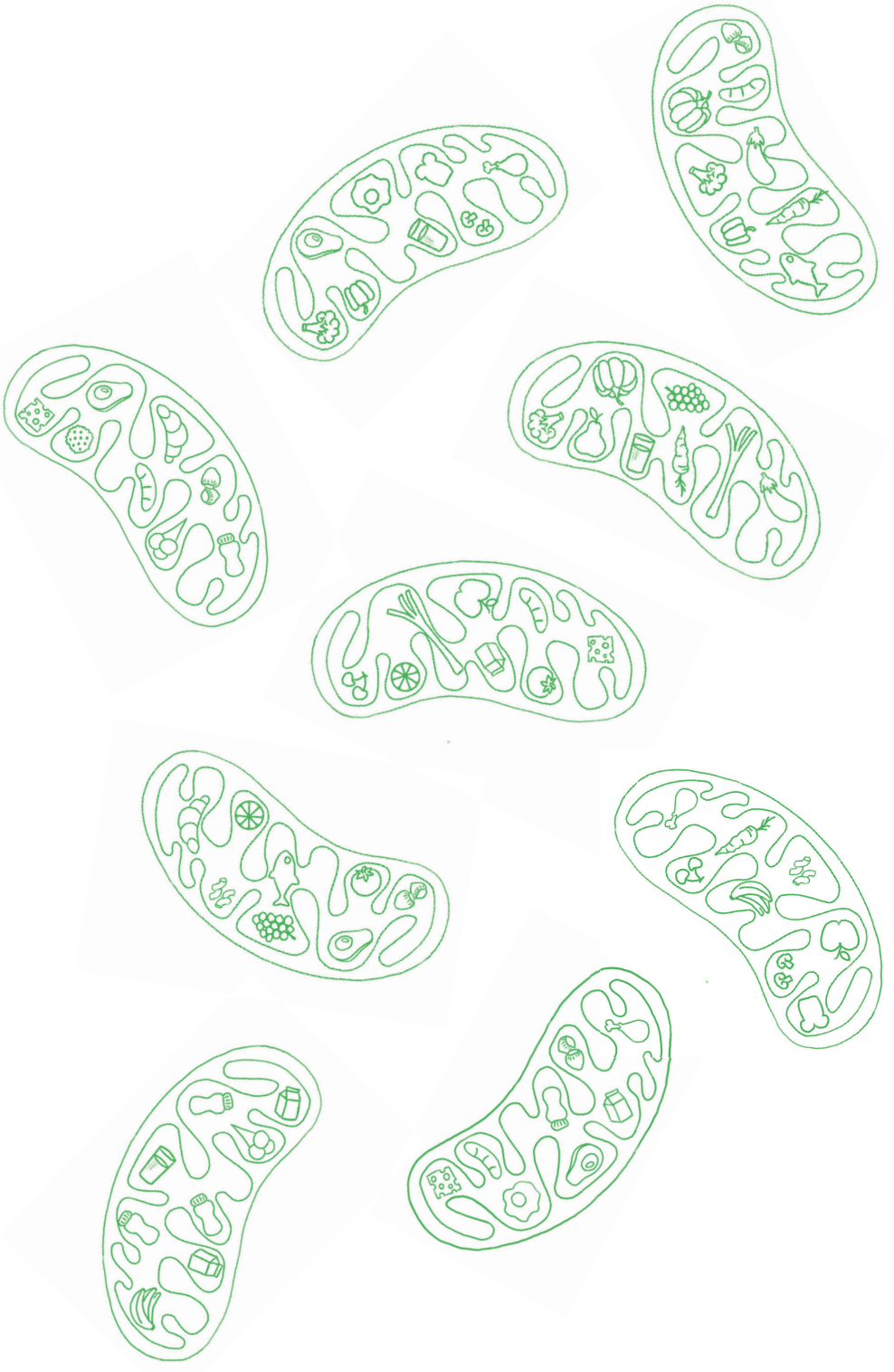

APPENDIX

SUMMARY

Mitochondrial diseases (MD) are relatively frequently inborn errors of metabolism. MD are a clinically and genetically highly heterogeneous set of disorders, characterized by a disturbed intracellular energy metabolism. Since no curative treatment is available so far, the management of MDs remains supportive. As such, dietary interventions are part of the therapeutic treatment plan, yet the scientific evidence to support this is weak. Dietary interventions commonly aim to treat or prevent malnutrition, gastrointestinal problems or epilepsy. Malnutrition in MD is felt to be common although the true incidence is unclear. Because of the quite heterogeneous phenotypical character of the MD population recent literature suggests to perform nutritional assessment to assess the nutritional state. This advice is hampered however by the notion that neither the most optimal techniques to perform this evaluation nor their actual cutoff values in the setting of MD remain elusive. Also, it is difficult to provide solid scientific proof for positive effects of any intervention on clinical outcome measures in the setting of MD given the small number of patients and, again, the heterogeneous nature of the population. We therefore assumed that a personalized intervention approach in these patients might be more appropriate and successful. This thesis aims to improve the knowledge on nutritional status and its determinants (aim 1), to provide ideas on the optimal strategy of nutritional assessment (aim 2) and to bolster the evidence for individually tailored nutritional intervention regimes (aim3) and, ultimately, improve the functioning and quality of life of adult MD patients.

Part 1 focuses on Nutritional Assessment in adult MD patients.

Chapter 2 is an observational study that explores and characterizes the frequency and severity of gastrointestinal complaints including dysphagia in 92 adult patients carrying the m.3243A>G mutation in mitochondrial DNA using a validated questionnaire. Data were compared with those obtained in healthy controls. This study showed that dysphagia and gastrointestinal problems, especially constipation, are common symptoms in the total m.3243A>G carrier cohort and are not related to heteroplasmy levels in urinary epithelial cells (UEC), nor to disease severity. The severity of gastrointestinal problems as well as overall disease severity is associated with an increased risk for malnutrition based on their lower BMI.

Chapter 3 focuses on nutritional intake. In this observational, cross-sectional, retrospective study, 60 three-day nutrition diaries of adult MD patients were analysed and compared with the Dutch recommended daily allowance and the Dutch National Food Consumption Survey. This study demonstrated that patients with MD have an inadequate diet. Specifically, intake of protein, calcium, dairy products, and fluids were low. Interindividual differences were found to be high.

Chapter 4 comprises the results of the DYNAMO study. In this two-site cross-sectional study, genetically proven adult MD patients were age-, BMI-, and gender-matched

to healthy controls. The results suggest that muscle strength is related to body composition and protein intake in MD patients. Seventy-three percent of MD patient were malnourished according to the GLIM criteria and/or PG-SGA, and 14-27% could be classified as sarcopenic. BIA overestimated fat-free mass when compared with Dual-energy X-ray absorptiometry (DXA).

Chapter 5 aimed to identify the optimal method to estimate total energy expenditure in MD patients. Resting energy expenditure was measured in adult MD patients carrying the m.3243A>G mutation using indirect calorimetry and was compared with results of 21 predictive equations for resting energy expenditure, as well as with indirect calorimetry data in healthy controls. Physical activity level (PAL) was assessed using accelerometery (SenseWear®) and compared with a fixed average PAL as well as with patients' self-estimated activity levels. Total energy expenditure was lower in MD patients than the recommendations for healthy adults because of their lower physical activity. In MD patients, 6 prediction equations for resting energy expenditure provided a reliable alternative for Indirect Calorimetry, with an accuracy of 71%-76%. The patients' own estimations for physical activity levels proved not to be a suitable alternative to accelerometery.

Part 2 focuses on Nutritional Interventions in MD patients.

Chapter 6 describes the results of the DINAMITE study. The aim of this randomized controlled trial was to explore the effect of an individually tailored dietary intervention on personalized goals, body composition, functioning, and quality of life in 39 adult MD patients due to the m.3243A>G mutation. The intervention group (n = 20) received an individually tailored dietary intervention over a 6-month period, whereas controls (n = 19) received standard care during 6-months (control period), followed by an individually tailored dietary intervention for the next 6 months (intervention period). Nutritional assessment and QoL measurements were performed at 3-month intervals. After 3 months of dietary intervention, 57% of the personalized goals were achieved. Most successfully realized goals were improved body composition, handgrip strength, and diminished gastrointestinal complaints. Consistent effects on functioning included improved handgrip strength, vitality, and fatigue score. These effects, however, did not seem to last after 3 months.

Chapter 7 provides a systematic review on the efficacy and safety of the ketogenic diet in genetically proven MD patients (both paediatric and adults). Data on efficacy and safety of KD for MD is too scarce for general recommendations. KD should be considered in individuals with MD and therapy refractory epilepsy, while it is contraindicated in mitochondrial DNA deletion(s) related myopathy. When considering KD for MD the high rate of adverse effects should be taken into account, but also spectacular improvements in individual cases. KD is a highly individual management option in this fragile patient group and requires an experienced team. To increase knowledge on this -individually-promising management option more (prospective) studies using adequate outcome measures are crucial.

A general discussion and suggestions for future research are described in **Chapter 8.**

In conclusion, malnutrition is very common in MD, even in patients with a normal or high BMI. Unfortunately, usual screening tools lack sensitivity to detect malnutrition in this chronically ill patient group. All adult patients with MD should receive full nutritional assessment of all three domains 1) food intake and requirements; 2) body composition; 3) functional parameters. This Nutritional assessment should include adequate interviews checking for gastrointestinal complaints, accelerometry, assessment of body composition (e.g. DXA or BIA with waist circumference) and functional testing (e.g. handgrip strength measurements and/or 30 second sit to stand test). Nutritional interventions should be tailored in a personalized manner and based on the nutritional assessment outcomes. Nutritional interventions can be helpful for MD patients to achieve personal goals in body composition, functioning and decreasing fatigue.

NEDERLANDSE SAMENVATTING

Mitochondriële ziekten (MZ) zijn relatief frequente voorkomende aangeboren stofwisselingsstoornissen. MZ zijn klinisch en genetisch zeer heterogene aandoeningen, die allemaal worden gekenmerkt door een verstoord energiemetabolisme. Aangezien er geen curatieve behandeling beschikbaar is, is de behandeling van MZ ondersteunend. Dieetinterventies vormen hier een belangrijk onderdeel van. Het bewijs voor deze dieetinterventies is echter schaars. De dieetinterventies bij MZ zijn veelal gericht op het voorkomen van ondervoeding, maag-darm klachten of epilepsie. Ondervoeding komt vaak voor bij MZ, maar hoe vaak precies is onduidelijk. Vanwege het zeer heterogene karakter van de MZ-patiëntenpopulatie wordt in de recente literatuur aanbevolen om Nutritional Assessment (het systematisch beoordelen van de voedingstoestand en voedingsbehoefte) uit te voeren om de voedingstoestand te bepalen. De meest optimale Nutritional Assessment technieken, noch hun afkapwaarden voor MZ zijn bekend. Het is moeilijk om wetenschappelijk bewijs te leveren voor positieve effecten van een interventie bij MZ vanwege het kleine aantal patiënten en de heterogene aard van de populatie. Daarom is een gepersonaliseerde interventie wellicht geschikter. Het doel van de studies in dit proefschrift is om de kennis over de voedingsstatus en de determinanten ervan (doel 1), de optimale strategie voor Nutritional Assessment (doel 2) en het bewijs voor individueel op maat gemaakte dieetinterventies (doel 3) te verbeteren en daarmee het functioneren en de kwaliteit van leven bij volwassen MZ-patiënten te verbeteren.

Deel 1 richt zich op Nutritional Assessment bij volwassen MZ-patiënten.

Hoofdstuk 2 is een observationele studie waarin de frequentie en ernst van maag-darm klachten bij 92 volwassen patiënten met de m.3243A>G-mutatie werd onderzocht met behulp van een gevalideerde vragenlijst. De gegevens werden vergeleken met die van gezonde controles. De resultaten tonen aan dat slikklachten en maag-darm problemen, vooral obstipatie, veel voorkomende symptomen zijn in het totale cohort van m.3243A>G-dragers en dat het voorkomen van deze klachten niet gerelateerd is aan heteroplasmie percentages of de ernst van de ziekte. De ernst van maag-darm problemen en de algehele ernst van de ziekte waren geassocieerd met een verhoogd risico op ondervoeding op basis van een lagere BMI.

Hoofdstuk 3 richt zich op de voedingsinname. In deze observationele, cross-sectionele, retrospectieve studie werden zestig driedaagse voedingsdagboeken van volwassen MZ-patiënten geanalyseerd en vergeleken met de Nederlandse aanbevolen dagelijkse hoeveelheid en de Nederlandse Nationale Voedselconsumptiepeiling. Deze studie toonde aan dat patiënten met MZ een inadequate intake hebben. Met name de inname van eiwitten, calcium, zuivelproducten en vloeistoffen was laag en de onderlinge verschillen waren groot.

Hoofdstuk 4 beschrijft de resultaten van de DYNAMO studie. In deze cross-sectionele studie met deelnemers van twee centra, werden genetisch bewezen volwassen

MZ-patiënten gematched met gezonde controles op basis van leeftijd, body mass index en geslacht. Dit onderzoek toonde aan dat spierkracht gerelateerd is aan de lichaamssamenstelling en eiwitinname bij MZ-patiënten. 73% van de MZ-patiënten was ondervoed en 14-27% werd geclassificeerd als sarcopeen. BIA overschatte de vetvrije massa in vergelijking met DXA.

Hoofdstuk 5 had als doel de optimale methode te identificeren om het totale energieverbruik bij MZ-patiënten te schatten. Het energieverbruik in rust werd gemeten bij volwassen MZ-patiënten met de m.3243A>G-mutatie met behulp van indirecte calorimetrie en vergeleken met resultaten van 21 voorspellende vergelijkingen voor energieverbruik in rust, evenals met indirecte calorimetrie gegevens van gezonde controles. Het fysieke activiteitsniveau (PAL) werd beoordeeld met behulp van de actometer (SenseWear®) en vergeleken met een vaste gemiddelde PAL en met het zelf geschatte activiteitsniveau van de patiënt. Het totale energieverbruik was lager bij MZ-patiënten dan de aanbevelingen voor gezonde volwassenen vanwege hun lagere fysieke activiteit. Bij MZ-patiënten boden 6 voorspellingsvergelijkingen voor energieverbruik in rust een betrouwbaar alternatief voor indirecte calorimetrie, met een nauwkeurigheid van 71% -76%. De eigen schattingen van de patiënten voor fysieke activiteitsniveau bleken geen geschikt alternatief voor actometrie.

Deel 2 richt zich op voedingsinterventies bij MZ-patiënten.

Hoofdstuk 6 beschrijft de resultaten van de DINAMITE studie. Het doel van deze gerandomiseerde gecontroleerde studie was om het effect te onderzoeken van een individueel op maat gemaakte voedingsinterventie op gepersonaliseerde doelen, lichaamssamenstelling, functioneren en kwaliteit van leven bij 39 volwassen MZ-patiënten als gevolg van de m.3243 A>G-mutatie. De interventiegroep (n = 20) kreeg een dieetinterventie op maat over een periode van 6 maanden, terwijl de controlegroep (n = 19) gedurende 6 maanden standaardzorg kreeg (controleperiode), gevolgd door een dieetinterventie op maat voor de volgende periode van 6 maanden (interventieperiode). Nutritional Assessment en kwaliteit van leven metingen werden uitgevoerd met tussenpozen van 3 maanden. Na 3 maanden voedingsinterventie werd 57% van de persoonlijke doelen bereikt. De meest effectieve doelen waren lichaamssamenstelling, handknijpkracht en maagdarmklachten. Een consistent effect op het functioneren werd aangetoond voor handknijpkracht, vitaliteit en vermoeidheidsscore, maar deze effecten hielden niet aan na 3 maanden.

Hoofdstuk 7 geeft een systematisch overzicht van de effecten en bijwerkingen van het ketogeen dieet (KD) bij genetisch bewezen MZ-patiënten (zowel kinderen als volwassenen). Gegevens over de effectiviteit en veiligheid van KD voor MZ zijn te schaars voor algemene aanbevelingen. KD kan worden overwogen bij personen met MD en therapieresistente epilepsie, terwijl mitochondriale DNA-deletie(s) gerelateerde myopathie een contra-indicatie lijkt te zijn. Bij het overwegen van KD voor MZ moet

rekening worden gehouden met het hoge aantal bijwerkingen, maar ook met spectaculaire verbeteringen in individuele gevallen. KD is een zeer individuele behandeloptie en vereist een ervaren team. Om de kennis over deze -individueel- veelbelovende managementoptie te vergroten, zijn meer (prospectieve) onderzoeken met adequate uitkomstmaten noodzakelijk.

Een algemene discussie en suggesties voor toekomstig onderzoek zijn te vinden in **hoofdstuk 8.**

Concluderend: ondervoeding komt veel voor bij MZ, zelfs bij patiënten met een normale of hoge BMI, en de gebruikelijke screeningsinstrumenten zijn onvoldoende gevoelig voor het detecteren van ondervoeding bij deze chronisch zieke patiëntengroep. Alle volwassen patiënten met MZ moeten een volledige nutritional assessment krijgen van alle drie de domeinen. Te weten 1) voedselinname en verbruik; 2) lichaamssamenstelling; 3) functionele parameters. Dit nutritional assessment moet interviews waarin maag-darmklachten worden nagevraagd, accelerometrie, meting van lichaamssamenstelling en functionele testen (bijv. handknijpkracht of een zit-sta-test) bevatten. Voedingsinterventies moeten op een gepersonaliseerde manier worden afgestemd en gebaseerd op de resultaten van nutritional assessment. Op deze manier kunnen voedingsinterventies voor MZ-patiënten nuttig zijn om persoonlijke doelen op het gebied van lichaamssamenstelling, functioneren en vermoeidheid te bereiken.

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Beste NAP collega's. Het NAP is ook al zo'n netwerk waar ik veel energie van krijg. Allemaal collega's zoals ik, met een passie voor het vak. Nooit te beroerd om klussen op te pakken, ik ben zo trots op wat we al bereikt hebben samen. **Hinke,** het is echt zo fijn dat jij ook lid bent geworden van het NAP. Jouw enorme kennis en ervaring met het zakboek en je link met het NTVD heeft enorm bijgedragen aan het bereiken van onze doelen. **Susanne Leij,** wij hebben samen het NAP opgericht en hoewel je nu zelf geen lid meer bent, ben ik je hiervoor enorm dankbaar. Onze samenwerking in de koppelstructuur was echt superfijn. Jij bent een beetje zoals ik, creatief, je denkt snel en je bent een doener. Jij hebt het DINAMITE acroniem bedacht en het NAP logo ontworpen en we hebben samen de DYNAMO studie gedaan. Ik mis je enorm en hoop dat je het naar je zin hebt in je nieuwe functie in mijn geboorteplaats.

Graag wil ik alle coauteurs bedanken voor hun bijdrage aan dit proefschrift. Van iedereen heb ik veel geleerd. Het begon met **Paul de Laat,** hij leerde me om, voor de structuur, kopjes toe te voegen die je later weer weg kan halen. Met **Saskia Wortmann** heb ik op kindergeneeskunde al samen gepubliceerd en zij is de laatste auteur van hoofdstuk 7 in dit boekje. Van de samenwerking voor dit artikel, hebben we allemaal erg genoten. **Saskia**, jij bent geweldig, supergetalenteerd en heel betrokken.

Mijn vele stagiaires wil ik enorm bedanken! Ze hebben bergen met werk verzet en zonder deze gratis arbeid was dit boekje echt nooit afgekomen. Ik heb ook echt genoten van het begeleiden van jullie en de gezelligheid en het talent dat jullie meebrachten. Er is één ex-stagiaire die ik nog even speciaal wil benoemen. Dat is **Elitsa Noom,** zij is nu mijn collega en ik heb echt enorm veel bewondering voor het traject wat jij bewandelt. Je hebt nu net in 1x je VWO wiskunde gehaald en kan aan je master beginnen. Je bent verder gewoon een echt leuk mens, altijd enthousiast en ik weet zeker dat wij vriendinnen blijven.

Lieve **Laila,** Ik ben heel blij dat jij mijn vriendin bent. Nu we beiden zo veel werken zien we elkaar minder vaak. Maar als we elkaar spreken, is het altijd gezellig. Dank je wel dat je het eten op mijn feestje wil verzorgen, zoals je ook al hebt gedaan toen ik 40 werd. Jij kan dat heel goed en ik hoop dat we nog vaak samen uit eten te gaan, nu dat eindelijk weer mag.

Lieve **Marieke,** wij zijn vriendinnen vanaf de lagere school en inmiddels zijn we vooral twitter vriendinnetjes vanwege de afstand tot Groningen. Weet je nog dat jij, ik en **Hanneke** elkaar lange brieven schreven via het plafond in de wc? Toen had je al meer talent voor schrijven dan ik. Ik wil je dan ook bedanken voor het corrigeren van dit dankwoord en voor het poepie dat we onze basisschool met onze publicaties laten ruiken!

Lieve **papa en mama,** dank jullie wel voor mijn opvoeding. Van jullie heb ik geleerd nooit op te geven en in jezelf te geloven. Ik vind het heel leuk dat jullie zo trots op mij zijn. **Mam**, ik ben ook heel trots op jou. Jij hebt vroeger, tegen het advies in de opleiding gedaan die je graag wilde en nu zorg je met zoveel liefde en geduld voor papa. Dank jullie wel!

Lieve kinderen: **Dylan** en **Kyra** maar ook **Karin** en **Mel** die er nu helemaal bij horen, ik ben zo trots op jullie! Vraag maar aan mijn collega's, ik vertel vaak over jullie. **Dylan** en **Karin**, dank jullie wel dat ik het feestje bij jullie mag vieren. **Mel**, bedankt voor het idee om bij iedere hoofdstukpagina een mitochondrie extra erbij te zetten. **Kyra**, jij bent de enige die nu nog thuis woont en dus heb je het meeste meegekregen van dit hele proces. Jij hebt waarschijnlijk zelf niet door wat een grote rol je speelt en hoe je me geholpen hebt. Jij vraagt altijd, als ik uit mijn werk kom, hoe mijn dag was en vervolgens luister je naar mijn antwoord over onderzoek dit en dat. Jij bent lief, gezellig, creatief en slim en ik kijk er naar uit dat we 31 oktober weer samen naar Duncan Laurence gaan.

En als laatste: lieve **Sylvester**, we hebben al veel meegemaakt in de 30 jaar dat we samen zijn. Jij hebt me gesteund op talloze manieren. Van het vrij nemen voor de kinderen als ik op congres was tot het regelen van een tweede monitor op mijn werkkamer. Jij hebt me laten voelen dat ik het waard ben om wat vaker voor mezelf te kiezen dan ik van nature doe. Samen hebben we twee fantastische kinderen opgevoed en nu genieten we van ons hondje **Droppie.** Ik hou van jou!

Heidi

CURRICULUM VITAE

Heidi Ester Emmy Zweers-van Essen werd geboren op 30 december 1974 te Utrecht. In 1992 behaalde ze haar HAVO diploma aan de HAVO-MAVO Presikhaaf te Arnhem. Aansluitend volgde zij de opleiding Voeding en Dietetiek aan de Hogeschool Arnhem-Nijmegen in Nijmegen. Vijfentwintig jaar geleden (1996) studeerde ze af. Met haar eindscriptie werd ze genomineerd voor de Novartis scriptie prijs. Nog voor ze haar diploma had werkte ze al als diëtist bij de thuiszorg Oost Gelderland en bij Voedingsadvies bureau Reduce. In januari 1997 begon ze als dietist in het Radboudumc. Ze werkte op de afdeling kindergeneeskunde waar zij zich specialiseerde in metabole ziekten onder leiding van prof. dr. R. Sengers. In 2010 stapte ze over naar de volwassenen metabole ziekten (Algemeen Interne Geneeskunde). Met onderzoek begon Heidi in 2004 met haar poster voor het SSIEM (Society for the Study of Inborn Errors of Metabolism) congres dat in Amsterdam plaats vond. De titel van die poster was “Biochemical evaluation and dietary treatment of recurrent hypoglycemic episodes in patients with mitochondrial dysfunction”. In 2014 werd officieel begonnen aan een promotietraject onder leiding van dr. Mirian Janssen internist metabole ziekten, dr. Geert Wanten, MDL-arts (co-promotoren) en Prof. dr. Joost Drenth, MDL-arts (promotor) dat resulteerde in het proefschrift dat nu voor u ligt.

Heidi was actief in het bestuur van de Nederlandse Vereniging van Dietisten, en ze was betrokken bij de oprichting van zowel de MODAZ (Nederlands netwerk voor metabole diëtisten) als de SSIEM-DG (Europees netwerk van metabole diëtisten). In 2013 ging Heidi samenwerken met dr. Susanne Leij, docent aan de HAN, in de koppelstructuur rondom het thema Nutritional Assessment. Zij hebben samen het Nutritional Assessment Platvorm (NAP) opgericht (nutritionalassessment.nl). De samenwerking binnen deze koppelstructuur heeft onder andere geleid tot gezamenlijk onderzoek dat beschreven is in hoofdstuk 4: de DYNAMO studie.

Heidi won de ESN (Erfelijke Stofwisselingsziekten Nederland) stimuleringsprijs in 2011. In Januari 2020 werd Heidi door het bestuur van het Radboudumc benoemd tot junior principal clinician voor haar werk als dietist metabole ziekten en het actometer project. In datzelfde jaar ontving ze de Nutricia Metabolics Research Fund Award.

Heidi woont in Elst met haar man Sylvester. Ze hebben 2 volwassen kinderen: Dylan en Kyra.

LIST OF PUBLICATIONS

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2021

Zweers, H., van Wegberg, A.M.J. Janssen, M.C.H. Wortmann, S.B., Ketogenic diet for mitochondrial disease: a systematic review on efficacy and safety. Orphanet J Rare Dis, 2021 16(1) p. 295.

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Wortmann, S.B., **Zweers, H**., Rasmussen, E., Rodenburg, R., Smeitink, J. A. M. and Morava, E. Investigation of mitochondrial mutations and haplogroup in Iranian patients with Parkinson's and Alzheimer's disease. Journal of Inherited Metabolic Disease, 2007. 30: p. 69.

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RESEARCH DATA MANAGEMENT

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The medical and ethical review board committee on research involving human subjects, region Arnhem-Nijmegen, Nijmegen, the Netherlands has given approval to conduct these studies. Published data generated or analyzed in this thesis are part of published articles and its additional files are available from the associated corresponding authors on request. To ensure interpretability of the data, all filenames, primary and secondary data, metadata, descriptive files and program code and scripts used to provide the final results are documented along with the data. The patient data for the analyses of studies as presented in Chapters 2, 3, 4, 5 , 6 and 7 is stored on the departments' shared drive: H:\Dietetiek\Algemeen\Onderzoek. All data from chapters 4 and 6 were anonymously collected and entered by use of Castor EDC (Amsterdam, The Netherlands). Data were later converted from castor EDC to SPSS (IBM, Armonk, NY, USA). The privacy of the participants in these studies is warranted by use of encrypted and unique individual subject codes. The code was stored separately from the study data.

PHD PORTFOLIO

Training activities	Year	ECTS
PhD 2.0: Digital tools for academic information	2012	0.3
BROK (NFU)	2012 2016 2020	1.5 0.5 0.5
Diagnostiek en evaluatie van de voedingstoestand: 3 day course on nutritional assessment (Pro education)	2013	0.8
Seminar Nutritional Assessment: PG-SGA (Hanze Hogeschool)	2014	0.3
Congress: Crossing borders, Innsbruck (SSIEM)	2014	1.0
Gebruikerstraining COSMED Quark RMR (TulipMed Academy)	2014	0.1
Diverse terugkomende bijeenkomsten : MDL journal club, RCMM research meetings, Radboud research rounds	2014-2020	3.0
Voeding in de Kijker (HAN) 3x	2015-2017	0.6
Schrijven van Wetenschappelijke Teksten (Radboud Universiteit)	2015	3.0
Statistiek voor promovendi (Radboud Universiteit)	2015	1.5
Congress: Healthy life trough nutrition, Lissabon (ESPEN)	2015	1.0
Academic Writing for PhD candidates (Radboud Universiteit)	2016	3.0
9e Nationale voedingscongres (interactie opleidingen)	2016	0.2
Presentation Skills for PhD candidates (Radboud Universiteit)	2016	3.0
Congress: Metabolic pathways, cellular networks and beyond, Rome (SSIEM)	2016	1.0
Digestive Disease Days, Veldhoven (NVGE)	2017	0.4
PhD student retreat (MDL, Radboudumc)	2017	0.5
Congress: International meeting on mitochondrial pathology, Keulen (Euromit)	2017	1.0
Congress: Clinical Nutrition and metabolism, Den Haag (ESPEN)	2017	1.0
Post HBO voeding en diabetes: 4 day course (HAN)	2018	1.7
Congress: Old roads, new connections, Athens (SSIEM)	2018	1.0
PhD student retreat (MDL, Radboudumc)	2018	0.5
Congress: Building bridges, Rotterdam (SSIEM)	2019	1.0
Virtual congress: DMIMD	2020	0.5

PHD PORTFOLIO

Teaching activities	Year	ECTS
Poster on international congresses: ESPEN, Euromit and SSIEM 20x	2004-2020	5.0
Supervision 24 (7x duo) bachelor students (HAN and Hogeschool v. Amsterdam)	2012-2020	17.0
Oral presentation SSIEM congress Birmingham 2x	2013	1.0
Oral ESN voorjaarscongres 2014	2014	0.5
Nutritional Assessment in de praktijk: workshop on PG-SGA (pt-Global)	2014	0.3
Diverse presentaties (10x) op Netwerkbijeenkomsten MODAZ, ketogeen samenwerkingsverband, NAP, pre rehabilitatienetwerk, vakinhoudelijke scholingen etc.	2014-2021	1.0
Supervision 15 master students (WUR and Radboud University and 1x ETH Zurich)	2014-2021	15.0
Workshop nascholingsdag ESN	2015	0.3
Oral presentation ESN najaar symposium	2016	0.5
Minor Klinische Voeding gastles stofwisselingsziekten (HAN) (3x)	2016-2018	0.6
Oral presentation ESN najaar symposium	2018	0.5
Workshop nascholingsdag ESN	2018	0.3
Minor Moving Questions Nutrition in mitochondrial myopathies Radboud Universiteit	2018-2020	0.3
Oral presentation 12e Nationale voedingscongres	2019	0.3
Oral presentation ESN najaar symposium	2019	0.5
Oral presentation International Conference, Leiden (TREAT-NMD)	2019	0.5
Oral presentation Mitochondria Symposium From Bench to Bedside (RCMM)	2019	0.5
Oral presentation virtual congress: nationale voedingscongres	2020	0.3
Module Nutritional assessment keuzemodule “voeding als medicijn” bachelor geneeskunde Radboud Universiteit 12 hours a course 4 x	2019-2021	1.7
Oral presentation virtual ESN voorjaarscongres 2021	2021	0.5
Oral presentation Belgische week van de ondervoeding	2021	0.3
Total		74.3

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